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REPRODUCTION IN THALLOPHYTES, WITH SPECIAL REFERENCE TO FUNGI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 393

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Introduction

LAVOISIER, in his *Traité Elémentaire de Chimie* (1793), wrote:

The impossibility of isolating nomenclature from science and science from nomenclature results from the fact that all science is formed necessarily of three things: the series of facts which constitute the science; the ideas which they call forth; and the words which express them.

Recently four major contributions (**44, 55, 59, 62**) have appeared which consider the problem of sexuality in Thallophytes. One of these, by KNIEP (**55**), is reviewed here with considerable restatement because it contains not only a wealth of factual material, but also statements of ideas in the form of working hypotheses, and a serious attempt to devise simple, precise, and consistent terminologies. This paper, in part, is an attempt to carry this clarification of ideas and terminologies a step further.[2] It is proposed to limit the scope of

[1] I am indebted to Professors E. J. KRAUS and H. E. HAYWARD of the Botany Department for many helpful discussions; and to Professor C. D. BUCK of the Department of Comparative Philology for help in the selection of terms. I am especially indebted to Professor SEWALL WRIGHT of the Department of Zoology for reading the manuscript, for giving me the benefit of his criticism, and for valuable suggestions. Thanks are also due my wife, Professor ADELINE DESALE LINK of the Chemistry Department, for helpful criticism and for aid with the literature and the manuscript. However, entire responsibility for the ideas expressed is assumed by me.

[2] A clarification of ideas relative to sexuality is proposed in a paper which has come to my attention since this article was in proof. ALLEN, C. E., Influences determining the appearance of sexual characters. Proc. Int. Congress Plant Sciences. Ithaca, 1926. 1:333–343. 1929 (see footnote 7).

the concept and term "sexual reproduction," or preferably to dispense entirely with it and its antithetic term "asexual reproduction." The paper also presents briefly the effect of recent genetic analysis on the concepts and methods of mycology and phytopathology.

The problem of sexuality in the Thallophytes has been a moot question since the discovery by MÜLLER (61) in 1782 of zygote formation in *Spirogyra*. It has played an important rôle in the motivation and ideology of three of the types of analysis applied to this division of plants. It was the spearhead of the studies of the comparative morphology and life histories of Thallophytes which have continued to this day in unbroken fruitfulness, as a result of the introduction of the technique of pure culture by BREFELD (11), and of experimental physiology by KLEBS (48, 49). It has been one of the main problems with which cytological studies have been occupied and to which some consider they have given a final answer. Latterly, since the rise of genetic studies, the problems of the phenomena associated with and conditioned by sexuality in Thallophytes have received a great deal of attention, with the idea that light might be thrown on genetic hypotheses, because of the phylogenetic position of the division. Consequently we find recent volumes by OEHLKERS (62) and by MORGAN (59), in their discussions of sexuality, sex inheritance, sex determination, and self-sterility, giving consideration to the researches and hypotheses of HARTMANN (43) on relative sexuality in algae, of JOLLOS (45) on relative sexual affinity in algae, and of KNIEP (51, 52, 53), BULLER (15), MOUNCE (60), BRUNSWIK (14), VANDENDRIES (74), and HANNA (40) on pluripolar sexuality of Hymenomycetes. The discussion by OEHLKERS (62) also utilizes recent data from fungi on the problem of the relative rôle of nucleus and plasma in inheritance in plants. In the main the extremely interesting data arising in such studies of Thallophytes have not materially changed the interpretations of geneticists who find it possible to fit the facts into explanations in vogue for higher plants. The attempt to fit the phenomena of relative sexuality of algae and of pluripolar sexuality of fungi into the mold of the self-sterility concept for angiosperms is a case in point.

It is evident from these discussions, and from others reviewed here, that neither the concepts nor the terms sex, sexuality, and

sexual reproduction are used in a precise and unequivocal manner. It also is interesting to note that in 1928 MORGAN (**59**) expressed the same misgivings, voiced by VAUCHER (**78**) in 1803, relative to sexuality in certain Thallophytes, because no morphological differentiation of male and female elements is present. MORGAN, referring to the so-called phenomenon of pluripolar sexuality in Hymenomycetes, states:

> Here we have the phenomenon of sex exhibited on a grand scale if we interpret the factors involved as sex factors in the conventional sense. . . . . It may seem of doubtful value to identify these factors with sex factors which conventionally at least apply to somatic differences in dioecious types or to those with separate sexes. It is true that amongst these differences are those concerned with producing eggs and sperms whose main function is to unite with each other, but, as generally understood, these functions are less conspicuous than those appertaining to the bodily constitution of male and female individuals.

Rôle of sexuality concept; genetic studies

Before considering in detail the confusion in ideas and terms relative to sexuality in Thallophytes, a great deal of which is inherent in the complexity, diversity, and intergradations of this, like all other biological phenomena, the significance of the concept of sexuality and the research which it has inspired and directed will be briefly discussed. The facts unearthed in the course of the studies of sexuality of Thallophytes and the ideas they have engendered have directly and indirectly led to significant theoretical and practical results, not only with reference to Thallophytes, but also to higher plants. Thus the foundations laid by KLEBS (**48, 49**) in his studies of the influence of environmental factors in the life histories of Thallophytes have been used by KRAUS and his associates and students (**56**) to arrive at facts and ideas whose application has profoundly modified horticultural and floricultural research and practices (**18**) in the United States. A comparative morphology of fungi by GÄUMANN (**37**) and GÄUMANN and DODGE (**38**), which is rational and does not lose itself in a morass of detail, has resulted from the utilization of the concept of alternation of nuclear phase as a *leitmotif*. The most far-reaching effects, however, especially since the rise of genetic analysis, have been experienced in the fields of mycology and phytopathology. The discovery of homothallism (monoecism) and heterothallism (dioe-

cism) as well as of the possibility of hybridization in the Mucorales by BLAKESLEE (6), and the discovery by BURGEFF (16) that "sex" characters are inherited independently like Mendelian characters in these fungi, have led to theoretical and practical consequences whose significance is just beginning to be exploited on a comprehensive scale.[3] As a result of studies for which these pointed the way, it is now realized that pure culture, in the sense of starting with a single spore or cell, the corner-stone of bacteriology, as well as much of mycology and algology, is not adequate in the study of Thallophytes, because heterothallic forms do not come to full expression. On the other hand, it is inadequate also, as pointed out by BRIERLEY (12, 13), because a single spore or cell may be heterozygotic. Thus, a smut spore and a sporidium from the promycelium of that spore may be quite different genetically. Consequently hybridization and other methods of the geneticist must become part of the technique of the algologist, mycologist, and phytopathologist, so that they may segregate the genotypes and effect new combinations. Tools have thereby been placed in the hands of the latter for attacking the problem of specialization and of biologic species of parasites, and to determine whether the phenomena involve: (1) modifications (Modificationen, Paravariation, in the sense of BAUR 3); Dauer-modificationen, in the sense of JOLLOS (variations which may persist for a long time but which are eliminated in caryogamy and reduction); (2) combinations (Kombinationen, Mixovariationen in the sense of BAUR 3); or (3) mutations (Mutationen, Idiovarianten in the sense of BAUR,

[3] Heterothallism has been experimentally indicated or demonstrated for the following Phycomycetes and Ascomycetes: *Dictyuchus monosporus* (COUCH 25); *Phytophthora faberi* (ASHBY 1 and GADD 36); certain Laboulbeniales (THAXTER 73); *Glomerella cingulata* (EDGERTON 35); *Ascobolus magnificus* (DODGE 32); *Penicillium luteum* (DERX 29); *Ascobolus carbonarius* (BETTS 5); certain Taphrinales (WIEBEN 80); and *Neurospora sitophila* and *N. crassa* (SHEAR and DODGE 68). The Basidiomycetes have proved an especially rich field for the application of the principles discovered by BLAKESLEE and BURGEFF. BENSAUDE (4) discovered heterothallism for Hymenomycetes in 1918; KNIEP (50) reported the same for Ustilaginales in 1919, and later for Tremellales (55); and CRAIGIE (26, 27, 28) discovered it for Uredinales in 1927. KNIEP (51, 52, 53) reported that copulation in Hymenomycetes is controlled by Mendelian factors; and BAUCH (2) reported the demonstration of secondary (physiological) sex characters for *Ustilago violacea*. KNIEP first (50) reported success in hybridization of smuts, not only between biologic races of *Ustilago violacea*, but also between species, such as *U. nuda*, *U. tritici*, *U. hordei*, and *U. bromivora* (54).

that is, variations which persist through caryogamy and reduction and represent true genotypes).

GOLDSCHMIDT (**39**), in a critical research, has applied himself to this question, in the hope of throwing light on the problem of specialization and on the more general problem of evolution of species. The research was designed to test critically the validity of the various hypotheses that have been advanced to account for the origin and nature of physiologic species. Biologic species have been taken to have their origin in (1) active adaptation of the parasite under formative influence of the host; (2) variation, induced by host influences (Dauer-modificationen); and (3) mutation and selection which lead to genotypically different races.

GOLDSCHMIDT used the biologic species of *Ustilago violacea* for this analysis. The reactions of the strains of the biologic species were tested against test stocks (A and a) to determine their "sexual reaction." With these tested strains, hybridization was carried out, and eleven hybrids of biologic races of *U. violacea* and six crosses between biologic races of this fungus and other species of *Ustilago* were tested for pathogenicity. Discouragingly low incidences of infection were obtained. Of the twenty-five control tests with mixtures of sporidia of the opposite sex on their hosts, infection occurred in only six hosts. Out of the eleven expected hybrids, six were recovered in appreciable quantity. The reason for the low percentage of infection was not determined. Possibly immunity and virulence phenomena, or unfavorable environmental factors were involved. At times the pathogenicity of the hybrids was different from that of the parents. Thus one hybrid did not inhibit pistil formation in *Melandrium album* (♀) as did its parents. The promycelia of parents and hybrids were studied critically, since no difference was detected between spores and sporidia, the hybrid promycelia as a rule being larger than those of the parents. One hybrid promycelium exhibited the morphological characteristics of one parent. Another hybrid gave irregular "wild" germination. Back crosses of hybrids and both parents were made, infection tests were carried out with these, and studies made of spore germination in the recovered progeny.

GOLDSCHMIDT concluded that in the biologic races of *U. violacea:* (1) Dauer-modification occurred and that one of these

involved plasmatic alteration (possibly diseased plasma); (2) a mutant was found which probably is of plasmatic origin; and (3) specialization is independent of the plasma of the fungus, and the biologic races of *Silene saxifraga* and *Melandrium album* differ by one Mendelian gene which determines specialization. Haploid infection does not occur, but infection does occur when only one nucleus of the dicaryophase is specialized for the host. Dominance in the dicaryophase in the hybrid race (*Silene saxifraga*×*Melandrium album*) was not manifested, as was reported for *Schizophyllum* by ZATTLER (**82**). It is also contended that the recovery from one hybrid of the characters of both parents proves that the brand-spore contains factors for these, and that since fusion of sporidia is the only place where they can possibly come together, copulation of sporidia is a sex act and that the sporidia physiologically are gametes. LAIBACH (**58**) has recently contended that sporidial fusion in the smuts is the functional equivalent of cell fusions in germinating conidia of Ascomycetes. BOSS (**10**) contends that it should not be considered a copulation but a fusion.

This contribution by GOLDSCHMIDT promises to mark a new era in the study of specialization and of physiologic races in fungi. STAKMAN (**70**) has recently reviewed this subject, and it is evident that heretofore ideas relative to specialization have been based on infection experiments, or cultural criteria of a morphological or physicochemical nature, and on inferences based on a few successful hybridizations and so-called mutations in fungi.

This study also points an additional way to analysis of mutation which is reported to occur abundantly in the fungi (**70**), and for which a summary was given by CHODAT (**20**). The most recent reports are from CHRISTENSEN and STAKMAN (**21**), CHRISTENSEN (**22**), and from CHIH TU (**19**). These mutations have been interpreted as genotypes by some investigators (**65**), even though no genetic analysis was made. That true mutations occur in fungi has been amply proved by the researches of KNIEP (**53, 55**), BRUNSWIK (**14**), VANDENDRIES (**75, 76, 77**), and HANNA (**40**) for Hymenomycetes. In fact, these studies indicate that geographic races may be due to genetic mutations and production of multiple allelomorphs.

The studies of HARDER (**42**) throw light, not only on the relative rôle of nucleus and cytoplasma in heredity, but possibly also on

"mutations" and "saltations" reported in fungi. HARDER attacked this problem directly by removing one of the paired nuclei from clamps of *Schizophyllum commune* and of *Pholiota mutabilis* with a micro-manipulator, and then observing the behavior of the operated cells. It was demonstrated that the operated cells withstood the shock, and that sexuality, clamp and fruiting body formation, and other characters are controlled by the nuclei. Hold-over influences of the ejected nuclei were noted in the formation of pseudo-clamps for some time after the operation. The habit of *P. mutabilis*, at least, was found to be controlled by the plasma to a great extent. A great number of the "mutations," "saltations," and "fluctuations" reported for fungi are changes in the habit of the organisms under study. In the light of HARDER'S study these may be temporary or permanent changes due to cytoplasmic conditions. Genetic studies are necessary before a final interpretation can be attempted for most of such reports of "mutations." Changes in habit similar to those reported as "mutations" have been induced by ultra-violet irradiation of fungi by STEVENS (**71**), and by RAMSEY and BAILEY[4] from the Cooperative Laboratory of this Department and of the United States Department of Agriculture.

The recent reports by DICKINSON (**30, 31**) for *U. levis*, by GOLDSCHMIDT (**39**) for *U. violacea*, and the less conclusive report by STAKMAN and CHRISTENSEN (**69**) and the convincing report of HANNA (**41**) for *U. zeae*, substantiate the earlier reports of ZILLIG (**83**) for *U. violacea* and of KNIEP (**54**) for *U. avenae*, to the effect that in some fungi only the dicaryophase is pathogenic. These, together with the report of GOLDSCHMIDT (**39**) that pathogenicity for a definite host is determined by a genetic factor, are destined to influence the concepts of virulence and of susceptibility and resistance. It has been apparent for some time that these concepts need recasting.[5] Success in hybridizing species of fungi and obtaining hybrids that ran the full course of their life history is reported by DODGE (**33**) for the Ascomycete *Neurospora*. This is significant in the light of previous re-

[4] In press. BOT. GAZ. **89**:

[5] SHARP, working in this laboratory, discovered that variation in virulence in *Bact. phaseoli sojense* is correlated with a rough-smooth variation and with variation in other morphological and physiological characters. SHARP, C. G., Virulence, serological, and other physiological studies of *Bacterium flaccumfaciens*, *Bact. phaseoli*, and *Bact. phaseoli sojense*. BOT. GAZ. **83**:113–144. 1927.

ports of success in obtaining zygotes in Phycomycetes, and hybrids in Ascomycetes and Basidiomycetes, most of which, however, failed to germinate or to complete the life cycle.

Application of the principle that certain Hymenomycetes are heterothallic has been used by L. F. BUTLER of the Cooperative Laboratory of this department and of the United States Department of Agriculture, in solving the tangle of the identity of certain rots of apples, from which "sterile" mycelia were quite consistently isolated by him.[6]

In view of the practical and theoretical applications of ideas based on them, it is not surprising that the phenomena of sex in Thallophytes, to which KNIEP's entire volume (**55**) is devoted, have aroused such interest. In another volume, HARTMANN (**44**) considers these phenomena as a part of his general discussion of reproduction, fertilization, and sexuality in the chapter on "Form-wechsel." OEHLKERS (**62**) gives them much attention in his discussion of modern genetics as applied to plants, and MORGAN (**59**) takes up these topics incidentally in his discussion of sex determination.

Causality of sexuality

HARTMANN attempts a causal analysis of sex phenomena, and follows BÜTSCHLI and SCHAUDINN in postulating that sexuality is one of the characteristics of fertilization and that it is unconditionally associated with it. He uses the term sexuality as the antithesis of male and female, which consists in a "sexual difference," at least a physiological one. This difference is taken to involve sex substances (Geschlechtssubstanzen) and to be probably nuclear. In formulating his theory of sexuality he admittedly follows CORRENS (**23, 24**), postulating that every sexually differentiated individual and germ cell possesses complete male and female potentialities (Potenzen). Preponderance of one of these complexes, due to inhibition of the other, produces a male or female tendency (Tendenz) in the cell. HARTMANN postulates that varying male and female tendencies may be conditioned by different quantitative mixtures of the true sex substances, and when the requisite sexual tension (gradient) has been realized between two gametes, they may fuse (relative sexuality). Varying tendency (with equal potentialities) may be induced by

[6] In press. Jour. Agric. Res. **39**:

various external influences or internal developmental factors at different stages of development of the individual (phenotypic sex distribution and determination); or by special hereditary factors, sex determiners (realizators, differentiators), which are not the only factors, however, so that reduction division and fertilization (genotypic determination) play the decisive rôle in the distribution and determination of sex.

HARTMANN (**43**) contends that his experiments with *Ectocarpus siliculosus* furnish the necessary facts to give support to his hypothesis. In this alga isomorphic gametes are involved that behave anisogamously in copulation, which HARTMANN takes to be a criterion of sexual differentiation. HARTMANN'S material was strictly dioecious. The gametes designated as female come to rest sooner than the male. The rate and frequency of copulation between gametes of various plants varied so that some males were designated as strong or weak males, while correspondingly on the basis of rate of settling the female gametes were designated as strong or weak females. Some plants produced gametes which reacted equally strongly as males or females. The strong males copulated not only with all females, but also with weak males, while strong females copulated with all males and with weak females. These phenomena he designated as relative sexuality.

The theory of sex substances, which was cautiously advocated by BLAKESLEE (**7**) in 1920, and the theory of direct correlation between maleness and femaleness and quantitative differences in qualitatively different male and female substances as developed by HARTMANN (**44**), are interesting and simple ones. Their experimental analysis may carry one into what WILSON (**81**) referred to as "an inaccessible field of inquiry," and involves the isolation of the so-called "sex substances." Possibly no pair of single substances is involved, but rather states or conditions which may be either physical or chemical, or both, and different in various plants. Modern genetic interpretations would suggest that a condition or substance complex, rather than two single substances (sex substances) is involved. Although no data are available on the isolation of fundamental sex substances in the Thallophytes, an extensive literature deals with the physiological, biochemical, and physico-chemical characteristics of sexual individuals. While it is not contended that they cause the

sexes, these differences are reported to characterize them. In organisms in general, the male sex is taken to be characterized by an activity (motility) metabolism, and the female by a passivity (storage) metabolism.

Among fungi, especially the Mucors have been extensively used in such studies because they are taken to represent sex in its simplest forms. Beginning with KORPATSCHEWSKA (**55a**), the Mucors have been studied to determine whether the plus and minus strains show physiological characteristics other than fusion; and latterly possible biochemical and physico-chemical characteristics have been sought. The literature of this topic has recently been summarized by SCHOPFER (**67**). Following KORPATSCHEWSKA (**55a**), contradictory reports have appeared relative to the characteristic abilities of mycelia of plus and minus strains in the utilization of given nutrients at definite temperatures. Recently SCHOPFER (**67**) carried on an extensive piece of research with *Mucor hiemalis*, and reports that consistent differences, such as rate of increase in diameter, absorption, reaction toward toxins, presence of carotin, effect on bacteria, and possibly serological specificity, exist between the plus and minus strains. He also reports that the megacopulation-branch is characterized by an abundance of fat. BURGEFF (**16**) reports that characteristics which are considered sexual in Phycomyces are separated from the sexes in meiosis. ORBAN (**62a**), on the other hand, reports that linkage was maintained through meiosis. In many studies of the characters of plus and minus strains this essential test has not been applied to the material, with resultant loss in value.

SATINA and BLAKESLEE (**64, 65, 66**) report for the Mucors, which show not only dimorphism (**8, 9**) but also all gradations between extreme plus and minus strains, that quantitative biochemical differences exist between plus and minus individuals. They used among others the Manoilow test, by which relative oxidation and reduction are measured, and report that the plus strains are relatively more reducing while the minus are more oxidizing. JOYET-LAVERGNE (**47**) was unable to demonstrate a similar difference in the oxidation-reduction potential (rH) for the plus and minus strains of *Coprinus* spp. supplied him by VANDENDRIES, but reports success with the micro- and megagametangia of *Fucus vesiculosus* and *F. serratus*.

There is no agreement even among biochemists as to the reliability of the Manoilow and similar methods. BURGEFF and SEYBOLD (17), who tested among other plants the extract of *Phycomyces blakesleeanus* by the same methods, conclude that they did not obtain consistent differences, and after running careful tests of the methods themselves, question their validity.

Even if the methods are valid, and the reports of positive results are substantiated, these experiments do not establish that qualitatively different sex substances exist in the sense of HARTMANN'S and BLAKESLEE'S earlier hypotheses. At best, these are secondary sex characters, the accompaniments or results, rather than the causes of sexual differentiation. It would be rash, however, to assume that further research might not establish the existence of specific qualitatively different sex substances.

The fundamental assumption made by HARTMANN, that copulation is conditioned by some sort of *difference* in the copulants which sets up a tension relieved at time of fusion, is grounded in the general dictum that likes repel and unlikes attract. It has an analogy in certain chemical reactions in which a balancing of plus and minus charges is concerned. However, it is not necessary to assume that fusion of copulants depends on differences. If we turn again to chemistry for analogy, we find instances of union between identical entities, as for example the union of oxygen or fluorine atoms to form molecular oxygen or fluorine. The assumption here made is that two identical unstable atoms pass into a stable state by sharing common electrons. This seems to be the method of combination of the carbon atom which plays an important and characteristic rôle in life phenomena. Attraction of likes is also indicated by chromosome orientation and conjugation, as well as by gene arrangements on homologous chromosomes. It seems to be a factor in successful hybridization between closely related organisms, and in the failure of attempted hybridization of distantly related or unrelated organisms.

JOLLOS' (45) results with *Dasycladus clavaeformis*, in which a change in relative sexual reaction was obtained by placing gametes of one sex into a filtrate of a suspension of gametes of the opposite sex, led him to conclude that he had not altered the sexual tendency but

rather the sexual affinity of the gametes. HARTMANN apparently does not consider this as critical an experiment as do others; at least he does not discuss its implications.

Kniep's interpretation of sexuality in Thallophytes

KNIEP's book had its origin in notes which he made in the course of his extended studies and those of his students on conjugate algae, brown algae, Hymenomycetes, and other Basidiomycetes. The volume, which is permeated with a genetic atmosphere, is a veritable mine of facts, oriented by clear ideas, which in turn are expressed in terms chosen with discrimination so that mere verbalism is avoided. The treatment of the complex detailed subject matter is characterized by sharp differentiation between fact and hypothesis; data are critically reviewed; gaps are pointed out; working hypotheses for bridging and filling them are suggested. The treatment attains unity because the problems of sex determination and physiological sex differentiation are kept in the foreground, and constitute the real theme of the book. The high points in the book are the discussions of studies by KNIEP and his students. These indicate by contrast what must still be done before a general theory can be attempted for sexuality of Thallophytes. The volume is well illustrated, and diagrams are used extensively to clarify the discussions of alternation of nuclear phases and of generations. KNIEP distinguishes between alternation of generations and alternation of nuclear phase, and subscribes to the view that the concept generation is not applicable when a gamete or a zygote would have to constitute a generation. He does not subscribe to the contention that the concept alternation of generations can be used only in a dual sense, but is willing to look upon each individualized body, or each body which produces a distinct spore type, as a generation. Thus the diplo-biontic Floridiae are considered by him to possess two nuclear phases and three generations, gametophyte, carposporophyte, and tetrasporophyte. He states, however, that it is debatable whether the aecium and the promycelium in *Puccinia graminis* should be considered as generations, and chooses not to consider the promycelium a separate generation because he does not care to be compelled to consider an ascospore or the basidiospore of Autobasidiomycetes a separate gener-

ation. Naturally alternation of nuclear phase and of morphological form are not always correlated. KNIEP takes the stand that it is misleading to apply the term "spore" to gametes or to the immediate fusion products. There is no attempt to discuss purely phylogenetic problems. KNIEP incidentally states that he considers the ascus and basidium as homologous structures, the latter being derived from the former. Autobasidiomycetes are considered the simplest Basidiomycetes, with their holobasidia, and from them those with septobasidia are taken to be derived. The brand-spores of the Ustilaginales are considered to be basidia which become sclerotic in youth (sclero-basidia). KNIEP is quite confident that future research will prove conclusively that CLAUSEN'S findings and interpretations of the nuclear phenomena in the Ascomycetes are correct, and that there is no double fusion and reduction. The main body of the book is prefaced by an interesting historical survey of the vicissitudes of the sexuality concept for Thallophytes, and concluded with a masterly summary in which the author clearly and unequivocally defines his concepts and his nomenclature, which draws freely upon previous nomenclatures, but is a simplification of current terminologies. Some new terms are introduced. He also states his views on the theories of relative sexuality, pluripolar sexuality, and self-sterility.

KNIEP does not define the concepts sex, sexuality, or sexual reproduction, but points out that the last covers a series of individual processes which in the main are characterized as follows: The series begins with differentiation and maturation of elements, the copulants, which come together by chemotropism, chemotaxis, or chemomorphism, depending upon their constitution. The first step of the sex act is cytogamy, which is followed at once, or after a more or less protracted dicaryophase, by the second step, caryogamy. Caryogamy is followed by chromosome conjugation, and then reduction division brings the series to a close.

KNIEP considers that caryogamy and reduction division are the fundamental features of sexual reproduction, and that there are no other general criteria ("weitere allgemeine Kriterien der sexuellen Fortpflanzung gibt es nicht"). In making his point that copulation of "specially differentiated, uninucleate cells which fuse to form

zygotes," the gametes, is not the essential criterion of sexual reproduction, KNIEP points out that copulation is not restricted to gametes, gametogamy, but that it also occurs between gametangia, gametangiogamy, or between somatic cells, somatogamy. The common features of all of these, however, are caryogamy and reduction division. Granted this point, the nuclear phenomena in *Hypochnus terrestris* and *Puccinia arenariae* are sex phenomena of an autogamous type. Intergrades occur. Thus in *Saprolegnia* and *Collema* there are combinations of gametogamy and gametangiogamy; in *Ascobulus carbonarius* there is a combination of gametangiogamy and somatogamy. Gametes may occur as planogametes or aplanogametes. The copulants (gametes, gametangia, somatic cells) may be isomorphic or anisomorphic. Identical behavior of isomorphic copulants constitutes isogamy. KNIEP questions whether there is true isogamy from the physiological point of view. (As pointed out before, it is not necessary to assume that copulation is determined by differences, and if this be true, there is no reason for assuming that real isogamy does not exist.) According to KNIEP, anisogamy in its simplest form is characterized by divergent behavior of the isomorphic copulants while oogamy is the most advanced type of gametic anisogamy. Both isogamy and anisogamy may occur in gametogamy, gametangiogamy, and somatogamy. Isogamy and anisogamy are concepts which offhand apply to the phenotypic aspect of copulants. Differentiation of copulants into male and female may be determined by caryogamy and reduction division (genotypic), or in the course of development (non-genotypic). Haplogametophytic Thallophytes are grouped by KNIEP on the basis of the character of their copulants as follows: (1) Phenotypically and genotypically alike; examples among fungi are *Olpidium viciae*, *Sporidinia grandis*, and *Schizosaccharomyces octosporus*. (2) Phenotypically alike but genotypically different; examples among fungi are *Mucor mucedo*, *Phycomyces nitens*, *Taphrina epiphylla*, *Aleurodiscus polygonius*, *Schizophyllum commune*, *Coprinus fimetarius*, *Ustilago violacea*, and probably *Puccinia helianthi*. (3) Phenotypically different but genotypically alike; examples among fungi are *Monoblepharis*, most Saprolegniaceae, *Dipodascus*, anisogamous yeasts, *Polystigma rubrum*, *Pyronema confluens* and *P. domesticum*, *Ascodesmis nigricans*, and all

monoecious Laboulbeniales. (4) Phenotypically and genotypically different; examples among fungi are *Dictyuchus monosporus*, *Pericystis apis*, *Ascobolus magnificus*, and *Dioicomyces*.

KNIEP here uses the term "phenotypic" in the sense of appearance only (phaenotypisches Bild). It is debatable whether the restricted use of the term is a happy choice. Phenotypic is used by many to mean anything in the nature of a character, be it morphological or physiological. It is a question whether copulants can be strictly alike phenotypically in this sense when they are genotypically different. Type 2 of KNIEP'S list may therefore be merely a special case of type 4. Certainly *Taphrina epiphylla* shows phenotypic differences in the behavior of its copulating conidia, and *Ustilago violacea* in the physiologic behavior of its sporidia in various culture media.

Diplogametophytic forms (occurring only among the algae) show a diversity of sexual reproduction, and for the dioecious forms the mechanism of sex determination is assumed to be like that of some phanerogams, that is, a combination of homogamety and heterogamety. Because of the use by geneticists of the term heterogamety in contrast to homogamety, KNIEP adopts the term anisogamy in preference to heterogamy which has generally been used in contrast to isogamy. KNIEP is not entirely consistent in the use of the term "gametophyte." He insists on the sharp differentiation between gamete, gametangium, and soma, which seems to make for precision and clarity of concepts and terms, yet he takes the liberty with the term gametophyte of applying it to any soma which bears gametangia, or to somatic cells functioning as copulants, as is the case in most fungi. The term copulantophyte could be used to designate those somas which bear copulants other than true gametes. If the soma bearing any type of copulant is to be referred to as a gametophyte, then the clarity and precision gained by distinguishing between gamete, gametangium, and soma as copulants are entirely lost.

KNIEP also suggests the following classification of sexual reproduction based on derivation of the copulants: (1) autogamy: obligatory copulation of the elements of one individual which may be (a) cytogamous (cells of the same individual) or (b) acytogamous (nuclei

of the same cell); and (2) xenogamy, the copulants being derived from different individuals.

A discussion of parthenogamy (in Ascomycetes) leads to the question whether copulation takes place between two male or two female copulants. At this point KNIEP takes up HARTMANN'S concept of relative sexuality and the phenomena of pluripolar sexuality in Hymenomycetes. Originally KNIEP had designated as sex factors those which control matings of mycelia and which, he found, behaved like Mendelian factors. He assumed the presence of like factors prevented matings. In the light of criticisms of this use of the concept and term "sex factors," he adopts the suggestion of OEHLKERS (62) to assume that copulation factors are involved, and thereby gather the phenomena of relative sexuality, relative sexual affinity, and pluripolar sexuality under the one concept "copulation factors." In accepting this suggestion, KNIEP now refers to the phenomena in Hymenomycetes as a "pluripolarity of copulation determining factors." The genes occur as multiple allelomorphs, and in the tetrapolar type two pairs of such allelomorphs, located on two pairs of chromosomes, are involved. The fundamental assumption is made that like genes prevent copulation. His argument in favor of copulation factors is as follows: Strictly speaking, the conditions determining copulation of isomorphic copulants which are genotypically different must have their basis in differences of the physiological structure. These cannot be assumed to be anything but copulation factors, which at first need not be genes. Copulation therefore involves a minimum of two different factors, which however are not the same ones that elicit sex differences (sex factors). Later, these two sets of factors may become linked, thus giving rise to true anisogamy (anisogamety). Consequently, for *Ectocarpus siliculosus*, he postulates that the linkage of copulation-determining factors and of sex-differentiating factors is not rigid. He rejects HARTMANN'S hypothesis of sex factors, admitting, however, that it is a simple and consistent one.

PRELL (63), BRUNSWIK (14), VANDENDRIES (75), VON WETTSTEIN (79), OEHLKERS (62), JONES (46), and MORGAN (59) have suggested that the phenomena of pluripolar sexuality in Hymenomycetes may be a matter of self-sterility factors, and OEHLKERS has pointed out that copulation factors could just as well be called sterility factors,

and thus one concept serve for all the phenomena in algae, fungi, and flowering plants. KNIEP rejects these suggestions. He contends that the fundamental assumption necessary in this hypothesis, that is, that all haplonts of isogamous Thallophytes are monoecious in that they carry potentialities for both sexes, and that therefore pluripolarity in Hymenomycetes is merely an expression of self-sterility as in monoecious flowering plants, is unwarranted. He contends that there is no ground whatsoever for assuming that isogamous Thallophytes, even monoecious Hymenomycetes, possess sex potentialities, pointing out that he believes that he is justified in applying the extended concept "monoecious" to them because there is no genotypic or phenotypic segregation of the copulation factors.

At this point KNIEP seems to depart from his otherwise rigorously logical analysis. If he assumes that in isogamous forms only copulation factors are involved, and that these are not in themselves sex factors nor linked with them, then the reproduction following copulation of these copulants, it seems, should not be considered an instance of sexual reproduction. This, however, leads one to the necessity of denying the validity of KNIEP'S fundamental assumption, that is, that the only essential criteria of sexual reproduction are caryogamy and reduction.

He contends further that self-sterility phenomena in *Nicotiana* and *Veronica* are not identical with the phenomena of pluripolar sexuality, for in the former reactions between diploid and haploid somas are the deciding factors, union of the haploid somas taking place if given an opportunity; whereas in the fungi it is an incompatibility between haploid elements that is involved. There may be merit in this objection. On the other hand, if we assume that something like an immunological reaction is involved, then it is not unreasonable to assume further that such reactions may take place between diploid cells, between haploid cells, and between diploid and haploid cells, so long as each pair contains factors whose balance leads to a production of the same state or substance. Certainly it is not necessary to postulate that the same things are involved in all of these reactions; one thing may be involved in fungi, another in flowering plants.

VANDENDRIES (**76, 77**), on the basis of extensive and exceedingly laborious investigations on Hymenomycetes, especially *Coprinus*

micaceus, has drawn very significant conclusions from his remarkable results. These results are in part identical with those reported by KNIEP (**55**). VANDENDRIES proposes a working hypothesis to explain the dihybrid or modified dihybrid copulation within the mycelia derived from one sporophore of *C. micaceus* as well as the essentially perfect fertility, on the one hand, and the essentially perfect sterility on the other, between mycelia of spores derived from sporophores of different localities, both near and distant.

He accepts KNIEP's hypothesis that copulation between mycelia derived from spores of the same sporophore, and greater fertility between spores of different sporophores are due to sexual allelomorphic genes (KNIEP'S recent copulation-determining factors), and that these phenomena are the basis of the so-called geographic races of Hymenomycetes. KNIEP (**55**) had expressed the opinion that this sterility between spores of sporophores from different localities might be due to factors which determine sterility between closely related species, and that it might depend upon excessive differences of the copulation-determining genes, or upon secondary factors. VANDENDRIES independently developed the idea that factors are operative in sterility akin to those which determine sterility between species, but he postulates that these are distinct factors, designating them as "dominant genes" in contradistinction to the sexual genes of KNIEP, to which he refers as "Kniepian genes." These dominant genes are taken to be subject to qualitative and quantitative variation which leads to mutation. The presence of the same dominant gene guarantees the possibility of copulation provided that the proper Kniepian genes are present. The dominant genes mutate independently of the Kniepian genes, and thus it is possible for sporophores to arise, none of whose spores will copulate with those of a sporophore from another region. The quantitative and qualitative differences permit intergrades, so that the mycelia of spores of an intermediate sporophore may copulate with those of spores of two other sporophores which have entirely different dominant genes between each other. This hypothesis of dominant factors in addition to Kniepian genes has much to commend it. It is in line with current genetical thought on the extremely complicated problems of sterility and fertility of organisms which JONES has recently sum-

marized (**46**). Certainly it seems possible that in fungi different sets of factors may determine compatibility between spores of different species, of different races, of different sporophores, and of the same sporophore if different and even independent sets of factors, which have been designated as fertility, sterility, compatibility, and incompatibility factors, determine ability of pollen tubes to grow, and selection between gametes of different individuals, and between gametes of the same individual.

VANDENDRIES points out that he considers all sporophores with the same dominant gene as constituting a "geographic race," irrespective of whether they show typical dihybrid copulation (in *C. micaceus*) or perfect fertility; and those with a different dominant gene, and therefore showing complete sterility, as another geographic race. According to KNIEP, sporophores which are perfectly fertile or sterile with nearby or distant sporophores belong to different geographic races. It does not seem advisable to designate these as geographic races when the real criterion of both authors is copulation or non-copulation. Biologic races would be a preferable term.

The concepts monoecious and dioecious are extended by KNIEP to isogamous forms. Those which produce copulable copulants in one individual are designated as monoecious, while those in which copulable copulants must come from different individuals are considered dioecious. The terms have only a phenotypic significance. It is debatable whether it is wise to extend terms which have attained and held quite definite and different meanings for a long time, especially when it is questionable whether sex is at all involved.

As used by KNIEP, the concept apomixis includes the following phenomena: (1) gametic apomixis (gametic parthenogenesis and ephebogenesis); (2) gametangial apomixis, both of which may occur in isogamous and anisogamous forms; and (3) somatic apomixis.

KNIEP does not attempt to formulate a general theory of sexuality on the basis of the phenomena noted in Thallophytes. He questions whether sexuality can be causally explained on a single principle as attempted by HARTMANN. He is inclined to postulate that the polyphyletic origin of sexuality in the Thallophytes indicates that the physiological processes which lead to fertilization may be very diverse, and that a unifying explanation may lie in ecological con-

siderations. Since amphimixis undoubtedly leads to increased variability, provided different genotypes enter into the zygote, which is not the case in "haplo-monoecious" organisms, KNIEP queries whether alternation of nuclear phase may not be of significance for mutability (für Mutabilität). It might be better to say that it may prove that alternation of nuclear phase as such may play a rôle in association with mutability. Mutation might occur in nuclei of monoecious forms if a rather long interval elapsed between pairing and fusion of nuclei.

Proposed clarification of concepts and terms relative to reproduction and sexuality

As pointed out before, there is confusion in the sex terminology. The difficulty is in part due to the fact that the same concepts and terms are applied to different things and situations, and that they are used in a specific or restricted, as well as in a general sense, quite indiscriminately. Another source of trouble lies in the fact that biological phenomena successfully resist classification. It is being assumed in the following discussion that the concept and term sex is the fundamental original one, and that the concepts sexual and sexuality are derived from it. In other words, sexual means "of or pertaining to sex" and sexuality "the character of sex." Sex is here assumed to mean *differentiation of a specific sort* which developed in association with fusion of copulants and zygote nutrition, protection, and development; that is, differentiation of individuals who themselves or whose individualized products are characterized on the one hand by activity, and on the other by passivity (storage metabolism), and generally also by distinctive size or form or both.[7] This interpretation is in harmony with common usage. The concepts sex, male, female, are probably originally based on pheno-

[7] ALLEN (footnote 2) points out that three categories of characters have been designated as sexual: (1) those which "arose in adaptation to the needs, imposed by the occurrence of syngamy, for securing the contact of gametes and for ensuring the sustenance and protection of the zygote"; (2) those which favor, hinder, or regulate the occurrence of syngamy; and (3) those of a chemical nature reported to exist between male and female individuals. He proposes to limit the concept and term sexual to characters of the first category. We are in essential agreement on this score.

typic bipolar differentiation of diplonts, as seen in man and the higher animals, that is, animals without alternation of generations, and possibly (certainly to a much more limited extent) on phenotypic differentiation of the sporophytic generation of diplohaplonts as seen in some dioecious flowering plants (*Phoenix dactylifera*, *Populus pyramidalis*). It would seem then that originally the concept and term sex expressed the idea of differentiation of somas, which were designated as the man and the woman, the male and the female. From this original meaning the terms male and female were extended, as adjectives, to the products of these differentiated somas, so that for the biologist, the products, after demonstration that cells are the effective agents in these products, became sex cells, which were called male germ cells (sperms) and female germ cells (eggs) respectively. In the case of plants, the terms male and female were later applied to the gametophyte (pollen grain, CAMERARIUS 1694). Since fusion is the apparent function of the "sex cells," and the nucleus came to be considered the essential element in the sex cell, caryogamy and reduction finally have come to be considered the sex act, and the elementary criteria of sexual reproduction and even of sex. It is apparent that in this process of extension, the criterion of the concept sex changed from differentiation of individuals to fusion of cells. From this derived point another extension was made in a different direction, in the contention that whenever cells (nuclei) fuse to form a zygote, be they differentiated or not, since this is sexual reproduction, they are sex cells, and that therefore their producers, even though they or their organs are not differentiated, are sexual individuals, either of different sex or of mixed sex. Now if we grant that because fusion occurs in sexual reproduction, fusion is the essential characteristic of sex, and that all individuals that fuse are sexual, then logically there is every reason for referring to the phenomena described for *Ectocarpus siliculosus* as relative sexuality, and to those described for Hymenomycetes as pluripolar sexuality, and for speaking of plurisexual individuals and of four, twenty, or more sexes.

Unquestionably there is apparent merit in the contention of HARTMANN and KNIEP that caryogamy and reduction are the essen-

tial criteria of what is called sexual reproduction or fertilization, provided one grants the validity of the extension of terms already discussed. On this basis isogamy, even of isomorphic gametes as in the Hymenomycetes, and the extreme autogamy in such forms as *Hypochnus terrestris*, are examples of sexual reproduction (KNIEP). MORGAN (**59**), however, points out that it is exceedingly confusing to apply the concept sex "where the phenomenon is one of union or non-union of gametes" (as in *Ectocarpus* and *Dasycladus*) "rather than one of sex as commonly understood," and suggests that the concept and term "sex factors" apply to factors "which conventionally at least apply to somatic differences in dioecious types or to those with separate sexes." PRELL (**63**), in discussing the phenomenon of pluripolar sexuality in fungi, is inclined to the view that it is best to avoid the term sex, because the occurrence of more than two sexes runs counter to common experience and sense. BLAKESLEE (**6, 7**) sensed the difficulty, and while he considered the phenomena in Mucors to be sexual reproduction, he referred to the copulants as − and +, a useful substitute for the terms male and female. DICKINSON (**30, 31**) tried to avoid the difficulty of referring to three sexes in *Ustilago levis* by using the term gender instead of sex.

There is a way out of this dilemma of ideas and terms. If we define sex as the *differentiation* of organisms into male and female individuals, then sexual reproduction clearly is not just any reproduction involving fusion, but only that which follows fusion of sexually differentiated individuals. Restriction of the use of the term to this sense would avoid, for example, the necessity of referring to twenty sexes in a *Coprinus*. Even when one uses the term sexual reproduction in this restricted sense, but designates all other types of reproduction as asexual and vegetative propagation, not all difficulties have been removed. The term asexual used in this sense is so inclusive that it has little exact meaning. In fact, it seems that the customary classification of reproduction on the basis of *sex* is a mistake, since it places emphasis on a subsidiary phenomenon so far as *reproduction* is concerned. An attempt is made here to devise a classification which avoids the antithesis of sexual and asexual reproduction.[8]

[8] Originally in this article, the antithetic terms "caryogamic reproduction" and "acaryogamic reproduction" were used in the attempt to clarify the concepts sexual and

All reproductive processes can probably be grouped as amitotic and mitotic. In the Thallophytes, according to our present knowledge, amitotic reproduction occurs quite regularly in the Schizomycetes and in the Cyanophyceae, and less abundantly in some of the true fungi, such as the Saccharomycetaceae. It is premature to attempt a classification of amitotic reproductive processes. Mitotic reproduction, on the other hand, can with a fair degree of accuracy be divided into two classes: (A) reproduction which involves nuclear change, caryallagic reproduction; and (B) reproduction which does not involve nuclear change, acaryallagic reproduction. By nuclear change is meant change in nuclear relations of the cell, as formation of a dicaryon, as well as change in the nuclei themselves, such as shift from the 1n to the 2n condition. Since caryallagic reproduction results in populations of non-identical individuals, it can also be designated as "aclonogenic reproduction," and the latter, since it results in populations that are clones, as "clonogenic." The first class comprises all cases of so-called sexual reproduction and some cases of so-called asexual reproduction, while the second class comprises most cases of so-called asexual reproduction.

The following classification of mitotic reproduction is based on the course and type of nuclear behavior involved in the production of individuals.

A. CARYALLAGIC REPRODUCTION.—Reproduction with nuclear change; this does not lead to clone formation (aclonogenic).

1. Nuclear fusion is immediately followed by reduction

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asexual reproduction as applied in the Thallophytes, and to clarify sex terminology in the fungi, which appears in its most confusing form in such phrases as "quadri-sexual" and "twenty sexes." Upon consultation with Professor SEWALL WRIGHT of the Department of Zoology, after he had read this paper in manuscript, it developed that he had been concerned about the confusion existing in genetics with reference to the terms sexual and asexual reproduction. A joint attempt was then made to devise a terminology which would be acceptable to the geneticist. We started with his suggestion of using production of genetically similar or dissimilar individuals as a basis for classifying reproductive phenomena. The term acaryogamic reproduction was dropped because it covered too many diverse situations. The terms clonogenic (clone-forming) and aclonogenic (not clone-forming) reproduction were evolved by us, and a search was made for terms to distinguish the various types of reproduction under those concepts. Later the writer proposed the terms "caryallagic" (karyon, nucleus, +allage, change) and "acaryallagic" reproduction, and those subsumed under these.

(meiosis): *caryogamomiotic*[9] *reproduction*. This type of reproduction is illustrated by plants in whose life history reduction occurs in the zygote, leading to the development of a haploid body (most Chlorophyceae and fungi).

2. Nuclear reduction (meiosis) after no, one, or four equational divisions, is followed by nuclear fusion: *caryomiogamic reproduction*. This type of reproduction is illustrated by plants in whose life history reduction occurs in gametogenesis and fertilization leads to a diploid body (pennate Diatomaceae with one equational division between disjunction and fusion; Fucales with two equational divisions between disjunction and fusion in oogenesis, and five equational divisions between disjunction and fusion in spermatogenesis).

3. Nuclear reduction (meiosis) is not immediately preceded or followed by nuclear fusion: *caryomiotic reproduction*. This type of reproduction is illustrated by the so-called "asexual" reproduction in the life histories of plants with sporic or intermediate reduction. In these, according to the current terminology, the "asexual" sporophyte by reduction produces "asexual" spores which give rise to "sexual" plants, the gametophytes (*Laminaria*, *Dictyota* Bryophytes, Pteridophytes, and Spermatophytes). It is also illustrated by haploid parthenogenesis and ephebogenesis.

4. Nuclear fusion is not immediately preceded or followed by nuclear reduction (meiosis): *caryogamic reproduction*. This type of reproduction is illustrated by production of zygotes in the life history of plants with sporic or intermediate reduction. Here, according to the current terminology, the "sexual" gametophyte through the process of fertilization produces zygotes which give rise to "asexual" sporophytes. The examples are the same as those listed under caryomiotic reproduction, excepting haploid parthenogenesis.

5. Nuclear pairing which is not immediately followed by fusion precedes reproduction: *caryozeugotic reproduction*. This type is

[9] This and the following terms have been synthesized from karyon = nucleus, gamo = fusion, and meiosis = reduction; anomos = irregular, mitosis = equational division; cytos = cell, blastos = bud, genes = producing; zeugos = yoking, pair; lysis = loosening, separation. Nouns can be made from these terms by addition of the suffix -gony.

illustrated by some fungi in whose life histories cell fusion or nuclear differentiation (as in *Hypochnus terrestris*) leads to a dicaryophase. This does not involve change so far as the nuclei are concerned, but it does involve change in nuclear relations of the cell which are significant morphologically, physiologically, and genetically. Thus such dicaryophytes are often more virulent than the haploid phase, and show dominance phenomena where genotypically different nuclei are involved. Clear-cut caryozeugotic reproduction is illustrated by gametogamy in *Taphrina epiphylla*, *Tilletia tritici*, and other Ustilaginales; by gametangiogamy in various Phycomycetes; by somatogamy in aeciospore formation in *Phragmidium speciosum*, by uredospore formation in *P. potentillae canadensis*, and all other cases in rusts in which nuclear pairing directly leads to spore formation.[10]

6. Nuclear separation occurs in the dicaryophase before fusion occurs: *caryozeugolytic reproduction*. This type occurs in fungi in whose life history the dicaryophyte produces uninucleate (1n) individuals by separation of the paired nuclei. It is illustrated by the formation of basidiospores in *Kunkelia nitens*, and by formation of uninucleate oidia in *Ustilago levis*.

7. Nuclear irregularities occur during mitosis: *anomomitotic reproduction*. This includes a great diversity of phenomena during cell division which may play a rôle in mutation. Illustrations are unequal distribution of chromosomes, both as to number and kind; failure of chromosomes to separate after division; unequal changes in homologous chromosomes and genes.

B. ACARYALLAGIC REPRODUCTION.—Reproduction without nuclear change; this leads to clone formation (clonogenic).

1. Reproduction by cell division: *cytogenic reproduction*. This is illustrated by equational or ordinary cell division, production

[10] It is debatable whether every case of pairing of nuclei (caryozeuxis) which is not followed by immediate fusion and which leads to a binucleate mycelium should be classed here. In so far as a new generation is produced, it would be correct to do so. However, generally the concept reproduction implies the formation of a new and distinct individual. If one chooses to designate all these cases as caryozeugotic reproduction, then the term should be reserved for the first cell that is formed by division of the dicaryon, because further cell division and spore formation by this dicaryon and its descendants do not involve nuclear change, and are a type of acaryallagic reproduction.

of agametes, that is, zoospores, sporangiospores, conidiospores, etc., also diploid parthenogenesis and ephebogenesis.

2. Reproduction by budding and vegetative propagation: *blastogenic*[11] *reproduction.* Sclerotium production is an illustration among the fungi. It is debatable whether the acaryallagically produced multicellular spores of fungi should be considered cytogenic or blastogenic reproduction.

It is apparent that this system enables one to classify reproduction without resort to the concepts sexual and asexual. Sexual reproduction would occur only as a *special* case in caryogamomiotic, caryomiogamic, caryogamic, and caryozeugotic reproduction when sexual differentiation of the copulants or of their producers is involved. In other words, this classification enables one to stress differentiation and not fusion as the characteristic feature in sex. It also indicates that the usefulness of sex must be sought in something other than mere reproduction. Abandoning the terms sexual and asexual reproduction would avoid, for example, such inconsistencies as the contention that a sexually differentiated sporophyte (*Phoenix dactylifera* for instance) or the sexually differentiated organs of a sporophyte (floral organs of any flowering plant) *asexually* produce sexual generations, the gametophytes, which *sexually* produce the sporophytes, which are, however, referred to as asexual. The definition of alternation of generations also can be restated as any periodic or more or less irregular alternation between generations with any two kinds of reproduction. This removes the obligatory association of alternation of generations and alternation of nuclear phase.

The general term copulant proposed by KNIEP (**55**) can be retained, because in itself it does not connote sex. If one chooses to define a gamete as does KNIEP, caryogamic, caryomiogamic, caryogamomiotic, and caryozeugotic reproduction can be considered to involve reproduction following gametogamy, gametangiogamy, or somatogamy, which may be isogamous or anisogamous. If one chooses to designate as a gamete any cell whose nucleus fuses, or any nucleus which fuses with the nucleus of another cell or with

[11] Unfortunately the root "blastos" which means sprout, sucker, shoot, has in zoological literature been used in a derived sense, that is, as germ. As a general biological term, somatogenic might therefore be preferable.

another nucleus, then these types of reproduction, excepting caryozeugotic, can be considered coextensive with "reproduction following gametic union," a phrase used extensively by KRAUS (**57**), or as "gametic reproduction." It seems preferable, however, to differentiate between gametes, gametangia, and soma as copulants, and to indicate the time relationship between copulation and meiosis, certainly in fungi, and consequently the phrase "reproduction by gametic union" is not a substitute for caryogamic, caryogamomiotic, or caryomiogamic reproduction.

Copulation and sterility factors can be hypothecated for homothallic (monoecious) forms with isomorphic copulants without introducing the concepts sex, sexuality, or sexual reproduction. When the level of unequal or unlike copulants is reached, one can speak of micro- and megacopulants (micro- and megagametes in anisogamety), using form, size, metabolism, or behavior as criteria, without any sex connotations if one wishes. On the other hand, one may interpret this as the level at which the simplest type of sex emerges, because there is differentiation of copulating cells or organs. At the next level emerges differentiation of the gamete containers as micro- and megagametangia. This is followed by differentiation of the entire soma which bears the copulants, as for example, when true gametes are borne, of the gametophyte into micro- or megagametophytes. At this stage one can truly speak of sex, in the sense that there is differentiation as male and female of the individuals which bear the copulants. Finally, the differentiation may even extend to the diploid soma which produces the copulant or the copulant-bearing soma (gametophyte).

It appears then that sex is a special adaptation which becomes associated with reproduction relatively early in the phylogenetic series if one considers differentiation of copulants as the criterion of sex; and relatively late, if one considers differentiation of the copulant-bearing somas as the criterion (MORGAN **59**).

Rationalization of caryallagic and acaryallagic reproduction

Can sexuality be explained causally, as HARTMANN attempted? What is the significance of its association with reproduction? Caryallagic (aclonogenic) reproduction, especially as represented by cary-

ogamic and caryomiotic reproduction in combination, and by gamocaryomiotic, caryomiogamic, and caryozeugotic reproduction, is a means whereby organisms acquired the mixing of germ plasms, amphimixis, and exploited its advantages. Consequently it may be considered a special adaptation. Now amphimixis not only tends to level differences between individuals, but it also provides means for enhancement of individual differences as a result of new combinations of factors. In this way it seems on the one hand to provide stability, and on the other hand, plasticity and diversity within the species. In association with mutation it becomes a powerful factor for plasticity and diversity, and with isolation, of species formation. It would seem therefore, that a species with reproduction involving nuclear change would tend to show diversity, and that in a changing environment this would be of survival value.

Caryallagic reproduction seems to have arisen independently again and again in the Thallophytes. Its manifestation, in spite of great diversity, falls into fairly general patterns which appear in the various series. One pattern seems to be the attainment of intensive amphimixis; another, the prevention of intensive amphimixis. The former is attained by cytogamy and caryogamy ranging from union of the copulants that are genetically alike to union of those that are genetically different; the latter by the reverse series. One can construct, as PRELL (**63**) has done, a series ranging from fusion of sister cells derived from a haploid mother cell, haploid autogamy (automixis), through fusion of cells derived from the same zygote, endogamy (endomixis), to fusion of cells derived from different diploid soma, exogamy (exomixis). Various devices and processes that occur again and again are employed not only to make endomixis and exomixis possible, but even to render them obligatory. These devices may be phenotypic, as in anisogamety, heterothallism, or heterophytism (heterozygotism). In anisogamety there may be extreme differentiation of the microgamete for mobility and of the megagamete for food storage at the extreme end of the series. These devices may also be genetic in the form of Mendelian copulation or sterility factors and heterogamety. Considered in this fashion, caryallagic reproduction makes amphimixis possible and various special

adaptations, such as sex, which have become associated with it, foster amphimixis and resultant diversity, and possibly enhanced chances of survival.

EAST (34) has pointed out that if n number of variations occur in an organism reproducing caryallagically, then 2^n number of types can be formed as a consequence of fusion and reduction, whereas in an acaryallagically reproducing organism, only n number of types can be formed. Thus if ten variations occur in the latter, only ten types can possibly result, whereas the same number of variations in the former have a maximum possibility of 1024 types. This is the situation when caryogamomiotic reproduction occurs, the zygote being reduced immediately after its formation, as in many algae (Conjugatae, Chlorophyceae, and haplobiontic Florideae), and in some fungi (Phycomycetes).

Caryallagic reproduction has exploited the advantages of amphimixis even more extensively in those organisms in which there is an extensive development of a diploid soma. In some of these forms, fusion of nuclei is not immediately followed by reduction, or cytogamy is not immediately followed by caryogamy or by dissolution of the dicaryon. The advantage of this lies in the fact that, whereas 2^n number of types may result when immediate reduction occurs, 3^n number of types may arise if a diploid body results and mitotic divisions of the 2n nucleus or conjugate divisions of the dicaryon are inserted between the fusion of the nucleus and reduction, or between cytogamy and caryogamy and reduction, so that great numbers of gonotoconts (spore mother cells, asci, and basidia) may result from one fusion, each reduction in turn carrying potentialities of new combinations.

To put it in another way, such organisms have a better chance of exploiting all the possible types of variations than do those in which reduction immediately follows nuclear fusion. SVEDELIUS (72) has pointed out that a plant with n number of chromosomes in the haploid state, and consequently 2n number in the diploid state, will have 2^{n-1} number of possible different combinations of haploid nuclei, and that 2^n is the theoretically minimum number of reduction divisions necessary to realize all the possible combinations. Thus "a

plant with 10 chromosomes in the haploid nucleus, and consequently 20 in the diploid, can by reduction divisions so combine them that 1024 ($=2^{10}$) different haploid nuclei can be formed, and for this at least 512($=2^{10-1}$) different reduction divisions are required." Delay of reduction (caryomiotic in combination with caryogamic, and caryomiogamic reproduction) occurs in the algae, which are characterized by the greater abundance of forms and species and higher differentiation of the thallus (diplo-biontic Florideae and Phacophyceae, also pennate Diatoms). Delay of fusion of nuclei (caryozeugotic in combination with acaryallagic and caryogamomiotic reproduction) is most marked in those fungi which have the greater number of forms and species, and the highest differentiation of the thallus. In the Phycomycetes the dicaryophase is relatively brief. In the Euascomycetes, development of the fruiting body is either initiated by the processes of nuclear fusion, or it is preparatory thereto, the soma however being haploid. In the Basidiomycetes the fruiting bodies are part of the dicaryophase, and are formed preparatory to caryogamy and reduction. Even in forms with extreme autogamy, as in *Hypochnus terrestris* and some Gasteromycetes, it is conceivable that the increased number of nuclear fusions and reductions may affect combinations of the factors resident in the one nucleus whose division leads to conjugate nuclei.

We have seen that caryallagic reproduction is an arrangement whereby reproduction and amphimixis are associated, and is a means of obtaining diversity and plasticity which may be of survival value in a changing environment.

On the other hand, acaryallagic reproduction can be considered a means whereby reproduction and apomixis (in the broad sense of PRELL and the restricted sense of KNIEP) are associated, and the advantages of the latter exploited. This tends to reduce diversity and to perpetuate the strain unchanged. It would seem that in an unchanging or relatively stable environment this situation would be of value to organisms that find themselves adapted, for they do not run the chance of losing a favorable variation by reshuffling of factors as soon as this is realized, as they do in amphimixis. Elimination of the possibility of new combinations would also be of advantage,

because most variations and new combinations seem to be useless if not harmful. Consequently one would expect a variety of arrangements which make apomixis possible and obligatory, or which tend to decrease the intensity of amphimixis, or even to eliminate it, in organisms which possess the facilities for caryallagic reproduction. One of these is persistence of acaryallagic reproduction as in fungi. Another is the quantitative decrease of caryallagic reproduction and the corresponding or even greater increase of acaryallagic reproduction as in fungi. Another is a series wherein the intensity of amphimixis is progressively diminished as in fungi (Ascomycetes) with parthenogamy, and those with extreme autogamy in the soma, as in *Hypochnus terrestris*. Another is apomixis in the restricted sense of KNIEP, in the form of gametic, gametangial, and somatogenous apomixis, all of which are represented in the fungi to a marked degree.

Finally, the chances of survival of a species or race in an environment which is essentially fixed but which either changes slightly now and then or, as is the case in all environments, is likely suddenly to change appreciably, would seem to be best if it combines the advantages of acaryallagic reproduction with those of occasional caryallagic reproduction, or at least keeps the latter in reserve. This situation we find in many successful parasites and pathogens, such as fungi and insects, for example. This arrangement may be of significance in those fungi which are characterized by an abundance of physiological species, varieties, or races, and in which both caryallagic and acaryallagic reproduction occur; the former as an occasional process, thus serving to produce new genotypes in heterothallic forms, and the latter to propagate, spread, and conserve the genotypes which find themselves adapted to their hosts. This may be the situation in some of the Erysiphaceae and Uredinales. In case CRAIGIE's reports (**26, 27, 28**) of heterothallism for *Puccinia graminis* are substantiated, this fungus may be a case in point when its complete life history is realized, with its intense specialization of races, its great display of acaryallagic reproduction on diverse hosts, and the comparatively infrequent inauguration of caryallagic reproduction by the various strains on the common alternate host. On this

basis the greatest display of genotypes of *Puccinia graminis* ought to exist in those regions where an abundance of barberry and a great diversity of grasses are combined.

We thus achieve a rationalization of caryallagic reproduction and sexuality in Thallophytes, in that they appear to be factors which foster survival and evolution. Sex is only one, although an important means of exploiting caryallagic reproduction. Caryallagic reproduction, especially in association with sex, seems to play a rôle in producing adapted organisms by favoring plasticity, diversity, and differentiation. This is not a causal explanation, since rather than telling us what really conditions caryallagic reproduction and sex, it points out what they accomplish, and savors of teleology in that it imputes purpose.

But even from the ecological point of view the situation is not unequivocal, for together with organisms which reproduce caryallagically are others, apparently equally successful, which do not reproduce in this manner. If we measure success on the basis of ability to survive in diverse and trying habitats, certainly the bacteria and the lichens, which are either without caryallagic reproduction or without intense amphimixis, are as successful as are Thallophytes which have intense amphimixis.

In caryallagic reproduction and sex, we find two opposed and apparently contradictory tendencies at work. One leads to intense amphimixis, ranging from automixis to exomixis; and the other to slight or no amphimixis, ranging from exomixis to automixis, or even to elimination of caryallagic reproduction, as in apomixis.

The pursuit of a single definite conclusion with regard to the causality of caryallagic reproduction and of sex will continue so long as curiosity concerning them exists, and will lead to ideas and experimentation with the promise of results of theoretical and practical significance. The pursuit may well prove endless, and we may have to say with WILLIAM JAMES, "There is no conclusion. What has concluded that we might conclude in regard to it?"

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DEVELOPMENT OF ANTHERIDIUM AND SPERMATOZOID IN PLAGIOCHILA ADIANTOIDES LINDB. (SWARTZ)[1]

DUNCAN S. JOHNSON

(WITH PLATES I–III AND FOUR FIGURES)

Introduction

This study of the development of the antheridia and spermatozoids of *Plagiochila adiantoides* was made upon plants which were found growing on decidedly moist soil in the damp passes or on the forested peaks of the Blue Mountains of Jamaica. It was from this island that this species was originally described by SWARTZ (LINDENBERG and GOTTSCHE 8). It occurs at altitudes ranging from 4500 to over 7000 feet. Shaded soil here may often be almost or even completely covered with this liverwort over areas of several square yards. The stems grow to 10 or 12 cm. in height, are only sparsely branched, and may stand erect or half-erect, or lie nearly horizontal. The plants of any one clump seem to spread by continually growing forward about the edges of the patch while dying off at the center or basal end. Wherever branching occurs, the death of the parent branch which follows two or three years later leaves the two branches as free, independent plants. This type of vegetative propagation, common among creeping bryophytes, evidently results after decades or perhaps centuries in suitable habits, in the formation of patches of hundreds or thousands of plants all descended from the one or the few pioneers that first established themselves at the spot. Clumps half a meter across may thus often be formed, each of which consists, in so far as the plants are fertile, entirely of antheridial plants or entirely of archegoniate ones.

Sexual reproductive organs and spore capsules were not found

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on more than 10 per cent of the plants. In most patches, at least between April and July when the plants for this study were collected, the percentage of antheridial and archegonial plants together

FIG. 1.—*a*, photograph of tip of male plant from above, showing forked series of involucral leaves below and another distinct series on tip of each branch; at base and along middle of each branch are sterile photosynthetic leaves; ×2.6; *b*, view from above of twice-forked tip of antheridial plant, showing structure of normal photosynthetic leaves below, and others, above lower antheridial spikes, reduced to half normal size. This alternation of groups of normal and fertile leaves on same axis is of frequent occurrence. Antheridia have been discharged from all involucral leaves except those at very tips of spikes; ×3.

was decidedly less than this. The plants used for this study were collected in various habitats in the Blue Mountains of Jamaica, on Blue Mountain Peak (7000 ft.), or at Morces Gap (4943 ft.) and New Haven Gap (5400 ft.) near the Cinchona Botanical Station.

FIG. 2.—Thrice-forked antheridial spike seen from above, showing cellular structure of sterile leaf, half urn-shaped form of involucral leaf, and position and relative size of antheridia; ×10.

They were fixed at various hours of the day, from 7:00 A.M. to 10:00 P.M., some in a medium chromoacetic fixing fluid and some in normal Flemming's fluid, and still fewer (1926) in formol-alcohol-acetic. In some cases fixation was done in the field. More commonly the plants were carried back to the laboratory in a tin box and then either fixed the same day or kept overnight in large, covered glass dishes, to be fixed early the following morning. After the return to Balti-

more the material was imbedded in paraffin, sectioned and stained in Flemming's triple or with Haidenhain's iron haematoxylin.

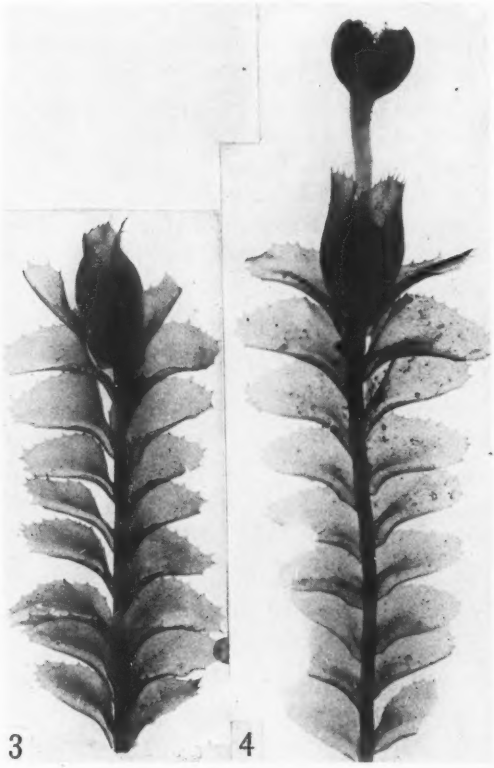
I. Vegetative structure

The vegetative plants of *Plagiochila adiantoides* have rather straight, rigid, often reddish stems which branch only infrequently in the free, exposed parts. Deeper down in the mat or turf of radiating, overlapping shoots, the forkings which give rise to the many free aerial shoots can be found. These terminal aerial branches may grow to a decimeter or so in length without branching. They are strikingly flattened and dorsiventral, and each bears from 40 to 100 markedly toothed, succubous leaves, which range in length from 3 to 5.5 mm. and in width from 2 to 4 mm. (figs. 1, 4). Rhizoids are never abundant. Those found are located at the bases of the frayed-out leaves, at least 10 or 12 cm. back from the apex of the branch bearing them. No rhizoids at all were seen on exposed parts of the stem which bore still functional leaves. It seems clear, from the sparsity and the small size of the rhizoids here, that this liverwort must take up most of the water and salts needed through the leaves themselves. The relatively large leaves of this species are usually pale green or yellowish in color. They are rarely as deep green as *Scapania nemorosa* becomes when moist.

II. Antheridial plant

The antheridial plant, or at least the fertile, aerial branches of it may, even in its still vegetative portion, be rather more slender than the sterile plant, that is, have shorter and often narrower leaves, although male plants are not always smaller plants. The fertile antheridium-bearing portion of the male plant may be simple, and 10–30 mm. long (fig. 1*a*). In other vigorous plants the fertile apex of the male plant may fork once, twice, or even three times in rapid succession, and thus, with further equal growth of the several branches, may give rise to a fanlike terminal group of antheridial spikes (fig. 1). In still other cases one or more of the primary divisions of the spike may itself divide in the midst of the series of involucral leaves to two, thus giving rise to secondary spikelets (figs. 1, 2). The branching in all such cases, as is evident at the beginning,

is truly monopodial. Later in the development, however, it may come to appear dichotomous.



FIGS. 3, 4.—Fig. 3, tip of archegoniate plant from above, showing normal leaves below and involucre at tip with inclosed sporogonium; ×6. Fig. 4, tip of archegoniate plant, seen from above, showing opened involucre, fully elongated seta, and ruptured capsule with protruding elaters and spores; ×6.

Not infrequently the axis of a male spike, after developing a score or two of involucral leaves, may begin to form a series of often a dozen or a score of foliage leaves. These may then be followed by a second series of antheridium-bearing leaves on the same continu-

ous branch, and these in turn by another series of foliage leaves. Whether both the fertile and sterile sections of the stem may be developed during the same growing season was not definitely determined. The foliage leaves developed above an antheridial spike are commonly not as large and vigorous as those of the basal part of the same shoot. In smaller plants they may be nearly so.

The antheridial spike may be of any length, from 10 to 25 or even 35 mm. In width it ranges from 1 to 2.5 mm. The transition from a vegetative branch to a male spike or the reverse may be quite abrupt, so that the limits of the two are clearly distinguished (fig. 1*a*). In other rarer cases the leaves of the vegetative portion of the shoot gradually become smaller through a series of two or three, or sometimes of twenty or more, leaves as we follow up the stem, until the antheridial branch has been reduced to only one-fourth or one-fifth the width of the foliage shoots, and the bases of the leaves have become deeply concave toward the stem (figs. 1*b*, 2). Antheridia are found only on such highly modified leaves, usually one in each axil. The transition from the antheridium-bearing to the normal foliage leaves, often found above them, may in like manner be either abrupt or more gradual (figs. 1*a*, *b*). The alternation of antheridium-bearing and sterile sections of the stem of the male gametophyte resembles strikingly the alternating sporophyll-bearing and sterile sections of the stem of the sporophyte in such club mosses as *Lycopodium lucidulum*.

The number of these involucral or antheridium-bearing leaves that may be formed in one continuous series on a single spike ranges from ten or twelve to thirty or forty, or more rarely even up to fifty or sixty leaves on each side. These leaves are borne in two ranks, one at the right and one at the left of the stem; that is, a spike of forty involucral leaves would have twenty of these on each side. These leaves are, of course, alternate in origin at the growing point. Sometimes they retain this distribution when mature (figs. 1*a*, 2, 3*b*); in other cases, however, successive involucral leaves of the right and left sides of the spike are almost opposite, thus forming apparent pairs. The leaves of such a pair may be separated by very short internodes, only one-fifth as long as the stretch of axis separating this pair from the next one above or below (fig. 2).

The antheridium-bearing leaf is not nearly flat, as the foliage leaf is except for its concave base, but the involucral leaf is curled upward from the dorsal (or upper side) of the stem, and then outward, next downward, then inward to the stem; and finally it turns downward again at the tip to form a protective structure with a half-urn-shaped base and a widely flaring margin. This margin is serrate, like that of the foliage leaf (fig. 2). The ventral edge of the involucral leaf extends vertically downward, so that these edges of the successive involucral leaves form a composite keel, four or six leaves thick, projecting from the lower side of the strongly dorsiventral spike (figs. 13, 16).

One antheridium only, with rare exceptions, is borne in the axil of each involucral leaf. In a very few cases two antheridia were present on one involucral leaf, where they had evidently developed successively. The younger antheridium in all such cases has a stalk that is inserted above, that is, nearer the stem apex than that of the older antheridium (figs. 9, 20, 36, 47). In none of the hundreds of cases studied were more than two antheridia present in the axil of a single leaf. It would be interesting to know the developmental sequence of the six or more antheridia shown by GOEBEL (5) to occur above each leaf in the antheridial spike of the related genus *Tylimanthus*.

The body of the mature antheridium is globular or slightly ellipsoidal in form (figs. 32, 34, 36). The length of its stalk is, for a long time, about equal to the diameter of the body of the antheridium. The stalk stands off at a sharp angle (45°) with the stem, and the body of the younger antheridium may often lie in contact, both with the inner surface of its own involucral leaf and with the outer surface of the next higher leaf (figs. 13, 36). A single cross-section of the younger part of an antheridial spike may pass through as many as four involucral leaves and two antheridia, or the stalks of these, on each side of the stem. The older antheridia often lie well above the level of the dorsal surface of the stem of the spike.

The persistent growth of the antheridial spike means that antheridia of many different ages may be found along its length. Thus in figs. 36 and 50 the basal antheridia are practically mature, while in the apical ones there visible the body and stalk are just being

differentiated. Still other spikes may bear only remnants of ripened and discharged antheridia below and very young few-celled rudiments above. The largest number of antheridia seen along a single, simple spike is twenty-two. These counts were made in preparations cleared in balsam or glycerin, and included all antheridia present from the smallest visible rudiment up to the most mature, still undischarged antheridium on both sides of the axis. In microtome sections where smaller rudiments can be seen, the total number discoverable in one spike may be 24–26. The number of antheridia that may be developed in a single growing season was not determined. In some habitats the growth of the plant may well be practically continuous, and antheridia may thus develop in regular succession all through the year.

III. Female plant

The archegoniate or female plant of *Plagiochila adiantoides* is commonly unbranched for a distance of 2–8 cm. back from the apex. The archegonial plants are frequently, although not constantly, distinguishable from the male by their greater robustness. The leaves of the female plant are unmodified except for the last two (one on each side) at the very tip. These two leaves are not expanded to right and left of the stem as those of the antheridial spike are, but remain pointing forward as all the leaves do when they arise in the bud. They are nearly vertical, rather than horizontal like the foliage leaves, but they are also strongly concave inward, which is likewise the form they came to have while still in the bud (figs. 3, 4). Although usually somewhat compressed from side to side, the involucral leaves do not fuse at the margins to form a continuous, cornucopia-like perianth as in many other foliose Jungermanniaceae.

The archegonial branch itself is always simple, so far as observed; never forked as the antheridial branches commonly are. Since usually but a single archegonium in each involucre is fertilized, there is but one sporogonium developed on each female branch. Two have been found in one involucre in not more than half a dozen cases out of hundreds seen. Since the archegonial involucre points straight out apically, a plane passing longitudinally through the axis, parallel to the leaves, would run through the group of archegonia.

IV. Development of antheridium

The antheridium initial, or mother cell, is often recognizable in the next to the youngest segment of the apical cell of the stem. The antheridium mother cell evidently arises from the acroscopic half of the stem segment, while the subtending leaf comes from the basiscopic half (fig. 8). The two walls first formed in the young antheridial rudiment are transverse. Of these, the first cuts off a basal cell (fig. 9), while the second wall, in the upper cell, separates a lower or stalk cell from a terminal or body cell (fig. 8). The first longitudinal wall in both the stalk and the body cell is a median or diametric one, which is usually approximately tangential to the upper right or upper left flank of the stem (figs. 8, 10, 13, 16). This first median wall usually makes an angle of about 45° with a horizontal plane, that is, with a plane lying parallel to the stem and through the antheridia of its opposite sides (figs. 13, 16, 20, 38, 43, 49). In fewer cases this wall may be more nearly vertical (figs. 39, 40) or horizontal (figs. 42, 48). In the stalk there is commonly no other longitudinal wall formed. Even the mature antheridium has a stalk which is still but two cells wide (figs. 17, 46).

The second longitudinal wall formed in each half of the young antheridium, the "quadrant wall," is often approximately radial, or in others more nearly periclinal. At its basal edge this is nearly perpendicular to the transverse or basal wall, which separates the antheridium from its stalk (fig. 24). This second wall cuts in two each hemispherical half of the antheridium (figs. 39, 43). This quadrant wall is often sharply concave, as is shown by the curved line (a quarter circle) in which it intersects the plane, median or diametric wall. It intersects the outer cell wall of the hemisphere in a less sharply curved line (figs. 8, 10, 27). The quadrant walls of the two halves commonly make approximately a right angle with each other (figs. 40, 41, 42). The second wall to be formed in each half of the antheridium is oftenest a very nearly periclinal one arising in the larger quarter (figs. 40, 41). The outer cell thus formed completes the protective antheridial wall of four cells in its circumference, and leaves a single, rather angular, half-dome-shaped primary spermatogenous cell in each half of the antheridium (figs. 20, 24, 41).

The further development of the wall of the antheridium results in the formation, by repeated anticlinal divisions, of a large number of protective cells in a single layer (figs. 23, 27, 29, 33*a*, 34, 48). These cells remain practically isodimensional until the antheridium is nearly mature (figs. 32, 35, 49). In the latest stages of development, while the radial and transverse dimensions of the cells of the wall remain about equal and constant, they often become appreciably elongated in the meridional direction (figs. 33*a*, 34). The total number of wall cells seen in cross and in longitudinal section of the mature antheridium is nearly the same, about 25–30. The inner and outer walls of these cells are only slightly different in thickness. Nevertheless they show a striking change in form at maturity, with the increasing turgor of the wall cells, similar to that which is known in antheridia of *Pallavicinia* and other liverworts (GOEBEL 4). The result of the greater stretching of the inner walls of these cells by turgor is the complete eversion of the several irregular flaps into which the upper half of the wall of the antheridium is torn when it bursts, until these flaps are bent outward and often downward toward the stalk of the antheridium. This is shown in fig. 35, which was drawn from a living antheridium in Jamaica in 1926.

The length of the capsule of the mature antheridium ranges from 0.2 to 0.4 mm. The length of the stalk is from one-half to three-fourths that of the body of the antheridium. The stalk shows no marked stretching at maturity, and the spermatozoids are therefore discharged within the cavity formed by the hollow involucral leaf. They must evidently make their way out (to reach archegonia) through the rather narrow crescentric slit between their own involucral leaf and the next leaf above this (figs. 13, 36). The mature spermatozoid within the androcyte finally becomes coiled to somewhat less than two complete turns (fig. 98). The maximum diameter of the body of the spermatozoid is about 1 μ, and its total length about 35 μ.

The later development of the single primary spermatogenous cell, formed as described in each half of the antheridium, may now be followed. The somewhat angular half-dome-shaped cell in each half of the antheridium (figs. 24, 41) is, before it has enlarged greatly, divided by an anticlinal wall. This first wall is often longitudinal

to the antheridium and practically perpendicular to the median, diametric wall (figs. 20, 42, 47). Transverse and other longitudinal and also diagonal anticlines and then periclines follow rapidly as the antheridium develops (figs. 11, 25, 26, 30). Later variously oriented walls appear all through the antheridium (figs. 31, 32, 33*a*, 49). Thus the two primary spermatogenous cells of the antheridium divide successively by walls in all planes, until there are from 700 to 800 "androcyte mother cells" present in the surface of a median longitudinal section of the antheridium and about 700 in a median cross-section. Since each androcyte mother cell forms two androcytes and then two spermatozoids, this of course means a total of some 25,000, to 30,000 spermatozoids from each antheridium (figs. 19, 33*a*). Before the final division each of these mother cells becomes rather cubical or somewhat polyhedral in form, and nearly isodimensional. The spindle of the final division in these cells more commonly, although not constantly, lies in one diagonal plane of the cube, and the plane of the resulting cell division thus lies in another diagonal (figs. 19, 82, 86).

The cytoplasm of all spermatogenous cells, from the beginning up to the young spermatozoids themselves, is rather finely vacuolated, and contains numerous very fine granules (figs. 51, 54, 55*a-c*, 70, 80). No plastids and no granules of distinctive size and form are at all constantly present, and no larger cytoplasmic inclusions were discoverable until the possible blepharoplasts, to be mentioned later, had appeared in the last mitosis. A larger, faintly staining, dark body (a limosphere?) sometimes becomes evident in the androcyte, after this mitosis (fig. 94*a*). The most careful search in hundreds of androcyte mother cells, in all stages of development, served to discover but few cases where anything comparable to a centrosome or blepharoplast could be clearly distinguished (WOODBURN 14). Even in those cases the minute, darkly stained body could not be distinguished until after the mitotic spindle of the very last division had already been organized; and then such a more pronounced dark dot could usually be found near but one of the two poles of the spindle (figs. 80, 82). Younger androcyte mother cells show a nearly spherical nucleus, with peripheral chromatin granules of various sizes in a more or less distinct reticulum (fig. 69). Slightly later

the chromatin thread thickens, its anastomoses become few, and then it breaks up to an at first inconstant number of loops and rods (figs. 71, 72, 73). The chromatin structure found in the resting nuclei of the preceding generation of spermatogenous cells (figs. 54, 63) can also be seen in very early phases of the androcyte mother cell nucleus. The nucleolus, usually present in the nucleus of earlier generations of androgones, as well as in that of cells of the wall of the antheridium (figs. 51, 54, 63) does not stain so distinctly in the nucleus of the androcyte mother cell (figs. 56, 57, 69).

Development of spermatozoid

The androcyte, as will be evident from what was just said of the form of its mother cell and of the position of the mitotic spindle in the latter, forms commonly a triangular prism. The base of each prism is one-half of one face of the cube, and the broadest face is that lying next to its sister androcytes (figs. 33*a*, 86). The cytoplasm of each ultimate androgone, "androcyte mother cell," just before its division, is regularly and finely vacuolated (figs. 63, 70). The cytoplasm of the daughter androcytes (young spermatozoids) is distinctly vacuolated at first (figs. 84, 86), but this commonly becomes somewhat less evident as development progresses and the protoplast elongates (figs. 58*a*, 86, 94).

The nucleus of the very young spermatozoid, when first organized after the final mitosis, is nearly globular (figs. 55*b*, *c*, 84, 86). The nuclear wall is distinguishable before the spindle fibers have disappeared, and remains clear until the developing spermatozoid has begun to coil (figs. 55*b*, 64, 87, 94). The chromatin of the young androcyte mother cell is found in rounded, or slightly elongated roughish grains (figs. 62, 63), which even at first are connected by but few visible strands of stainable substance (figs. 63, 70). The number of these grains, which differ markedly in size, is at least twice that of the chromosomes. Even these small grains seem composed of still more minute granules, as becomes more evident when the chromosomes for the last mitosis are organized (figs. 79, 83, 85). The nucleolus, which is evident in resting nuclei of all spermatogenous cells, up to the young androcyte mother cell (figs. 54, 62, 63, 69), disappears with the organization of the chromosomes at mitosis

(figs. 55, 72, 76). No clear evidence was obtained of any exchange of material between nucleolus and chromosomes.

The changes in organization of the nucleus of the androcyte mother cell before the final mitosis include a disappearance of many of the stainable connecting strands between the numerous chromatin grains. This is followed by the aggregation of all the chromatin into a finally definite number of evidently composite granular masses, which are later to become the chromosomes (figs. 69, 70, 73, 77). The smaller, more numerous grains (fig. 69) are often angular or irregular in shape, are peripheral in position, and are definitely connected to an irregular reticulum, in which they lie at the nodes. These grains, by their decided roughness, show some signs of being composite, but they are not evidently hollow, as the larger aggregations soon to be formed often are (figs. 71, 75, 79).

The next evident step in the progress of this last mitosis is the aggregation (the further collection) of the chromatin granules to form the rodlike and often hollow chromosomes. When first formed the latter are evidently composed of a number of granules each (figs. 73, 79); somewhat later the component granules have become less distinct, and the still oblong chromosomes seem to have a nearly continuous dark outer layer, with a less deeply staining central region (figs. 76, 78). This difference in staining capacity of the axial and peripheral portions of each chromosome persists, and is clearly evident whenever the stain is not too intense (figs. 76, 79). Not only is this true, but in well stained nuclei the peripheral chromatin can still be seen to be composed of grains which often seem to be grouped in transverse bands around the circumference of the chromosome (figs. 74, 79). In more deeply stained chromosomes these chromatin granules may be indistinct, or may even be quite indiscernible (figs. 75, 77, 78). In the chromosomes as grouped for the mitosis, from prophase to anaphase, the chromosomes have become shortened to about half their original length, and seem practically solid when stained to show at all clearly (figs. 77, 80, 82).

Many mitoses in spermatogenous cells were studied in search of any constant difference in size or form among the ten chromosomes of any individual nucleus, or differences (in size or number) between

the chromosome complements of different spermatozoids. Neither type of difference was discovered; that is, no differences were found in the chromosome complements of different spermatozoids that might be correlated with differences in sex, or in other qualities, in the individuals produced by eggs fertilized by these different spermatozoids (ALLEN **2**, SHOWALTER **13**, and MCALLISTER **9**). Unfortunately no meioses were found of spore mother cell nuclei with their probably larger nuclei and chromosomes.

At the equator of the last mitotic spindle in the spermatogenous cells the chromosomes are only slightly elongated, or may be nearly globular (figs. 77, 80, 82). No good examples were found of the groups of daughter chromosomes when first arrived at the poles. Slightly later, when the daughter (spermatozoid) nuclei have been organized, the chromatin is found in rather numerous, scattered roughish angular grains, arranged in a rather coarse reticulum (figs. 55*a*, 83, 84). At about this time or soon after, the chromatin granules may collect into (about ten) evidently composite masses of rounded form (figs. 55*a*, *b*, 81, 83). In fig. 85 the size and number of granules apparently "chromomeres" in each mass or chromosome are approximately constant, like the number of the chromosomes themselves.

From the time of separation of the young androcytes to the formation of a cell plate (but not of a definite cell wall) they are commonly flattened against each other, so as to look somewhat triangular in cross-section, that is, in a section parallel to the axis of the last spindle (fig. 86). This form may sometimes be due to the diagonal division of the cubical mother cell, but in other cases apparently to the subsequent flattening of the daughter cells against each other. The spermatid nucleus is at first globular and central in position, as is true of *Blasia* (SHARP **11**), *Polytrichum* (ALLEN **1**), and many other archegoniates. The chromatin granules are rather large and only sparsely connected into a reticulum. Later the nucleus begins to elongate, and the chromatin grains become connected up more abundantly, often in somewhat parallel beadlike chains (figs. 64, 87, 92).

It is at this stage of nuclear elongation also that the blepharoplast for the first time becomes clearly evident, as a darkly staining

rod on one side of the spermatid, beside the elongating nucleus (figs. 57, 86, 93). The blepharoplast of *Plagiochila* seems usually to be in actual contact with the nucleus from the beginning of elongation of the latter. Only rarely can it be seen to lie nearer the cell wall and to separate from the nucleus (fig. 97*a*), as it is shown to do in *Marchantia* (IKENO **6**), *Blasia* (SHARP **11**), and *Polytrichum* (ALLEN **1**). Earlier than this stage nothing can be found constantly in the cell that can be identified certainly as the progenitor of this dark rod which is later to bear the two cilia of the mature spermatozoid. A blepharoplast then does not become unmistakably evident at so early a stage here as IKENO, WOODBURN, ALLEN (**1**), and SHARP (**11**) have found it to do in other bryophytes. There is thus no evidence that the blepharoplast is derived from a persistent body having the characteristics of a centrosome.

In the later development of the spermatozoid the nucleus continues to elongate, usually becoming more slender at one end than at the other, and the whole nucleus thus comes to have a rather pear-shaped form (figs. 58, 65, 94*d*). This is apparently the characteristic manner of elongation of the spermatozoid nucleus in both bryophytes and pteridophytes (SHAW **12**, IKENO **6**, YAMANOUCHI **15**, ALLEN **1**, SHARP **11**). During these phases of development the chromatin granules of the nucleus often take a somewhat indistinctly beadlike arrangement, with the strings longitudinal to the nucleus (figs. 65*b*, 92).

When the spermatozoid has later become elongated to a length four or five times its diameter, the chromatin often has the form of six or seven transverse groups or bands (figs. 59, 60, 95*d*). Sometimes, although this is not always clear, these bands have a slant which suggests that one is really seeing successive turns of a single spiral which appears to reach nearly the whole length of the spermatozoid (figs. 61, 67, 95). Fig. 60*b*, of a cross-section of such a nucleus, shows that the chromatin band is peripheral and not complete in any one plane. In fig. 94*a*, which is that of a well advanced spermatozoid, only the barest suggestion of transverse bands of the chromatin is evident. In some few young spermatozoids of about this age, which were evidently well fixed, the whole nucleus seemed coiled (figs. 66, 95*c*, 96). In all cases where the relative ages could be de-

termined these coiled nuclei occurred at an earlier stage of development than the characteristic coiling of the whole spermatozoid in a single plane, which is shown in figs. 61, 88, 90, 94, 98. In some of these cases of the apparent coiling of the nucleus within the uncoiled cytoplast it seemed possible that the apparent coiling was due to the alternation of the bulk of the chromatin on the two sides of the elongated nucleus. In other cases, in slides where adjoining antheridia were well fixed, the independent interpretation of several trained observers agreed in regarding the whole nucleus as really spirally coiled, as is indicated in figs. 66 and 96. No adequate series of consecutive later stages was found, in spikes where this type of coiling of the nucleus occurred, that would allow following closely the further development of this sort of spermatozoid. This coiling may prove to be abnormal although it is not rare. It is hoped that further study of this and other species of liverworts will show its significance.

The further flat (watch-springlike) coiling of the spermatozoid nucleus seems to be accompanied by a gradual separation and disappearance of the transverse chromatin bands. This may be due primarily to a rearrangement of the chromatin granules composing the bands (figs. 67*b*, 90, 94*a*, *d*). As is evident from figs. 88, 90, 95*a*, *b*, however, the chromatin still retains its peripheral position in the nucleus. The chromatin of the elongated spermatozoid nucleus seems to form a sort of curved, tubular reticulum, along the outer side of which lies the blepharoplast. This peripheral position of the chromatin shows especially clearly in all moderately stained and lightly stained cross-sections of spermatozoids, from this stage up to the practically ripe ones (figs. 89, 94*b*, 99).[2]

The darkly staining blepharoplast that first becomes distinguishable when the spermatozoid nucleus begins to elongate, remains clearly distinct in most spermatozoids from this time until the spermatozoid is mature. At first the blepharoplast is rather short and

[2] It was evidently a series of peripheral chromatin granules similar to those referred to previously, which were seen in cross-section of the spermatozoid of *Monoclea*, complicated perhaps by the overlapping coils of the two spermatozoids of each pair, that led the writer (7) to a misinterpretation of this stage of the spermatozoid, in an earlier study of that liverwort. A reexamination of the slides shows that these stages of the spermatozoid may there be interpreted as they have been here in *Plagiochila*.

thick, one end being thicker than the other, and is but slightly curved (fig. 93). A spherical blepharoplast has not yet been found, unless the dot near the upper pole of the spindle in figs. 80 and 81 be so interpreted. Later the blepharoplast is attenuated to a length many times its diameter, and becomes curved to conform to the coiled spermatozoid, along the slender anterior end of which it lies (figs. 88, 94, 97). In most cases the blepharoplast seems to lie in close contact with the nucleus. In fig. 97*a* is shown one of the few cases where it is evidently separate throughout most of its length. With increasing attenuation the blepharoplast commonly remains practically homogeneous, although it may occasionally show slight irregularities in thickness (figs. 88, 92). It seems to be always continuous and never vacuolated or clearly fragmented, however, as SHARP (11) has shown it to be in *Blasia*, and as it had earlier been found to be in certain pteridophytes (SHAW 12) and cycads (CHAMBERLAIN 3). The appearance shown in fig. 90, where the coiled spermatozoid has a beadlike outer border, seems clearly due to the granules of peripheral chromatin and not to any discernible fragmentation of the blepharoplast.

The ripe spermatozoid has a slender body, cylindrical except for its tapering anterior fifth. It is coiled to about one and a-half or one and two-thirds turns in a flat spiral. Its two cilia are about one-third the length of the body itself. The cytoplasm of the body of the spermatozoid is but slightly granular, while that of the rest of the androcyte shows no appreciable differentiation, except usually a rather globular, granular mass (limosphere?) near the center of the coil (figs. 98, 99). When the living mature spermatozoids are discharged into surrounding water drops by the irregular bursting of the wall of the antheridium (fig. 35), they are almost transparent. As the spermatozoid swims about, its flat spiral commonly becomes drawn out to a rather corkscrew-like form. The entrance of the spermatozoid to the archegonium and the actual fertilization of the egg formed in the latter organ have not yet been observed. It is expected that such stages of the development of the sporogonium as have been seen will be described in a later paper, when sporogenesis has been studied more completely.

Summary and conclusions

1. This study was undertaken primarily to discover the formation and detailed structure of the very striking antheridial spikes of this liverwort, the development of antheridium and spermatozoids, the origin and organization of the blepharoplast, and the occurrence or non-occurrence of sex chromosomes.

2. The antheridial spike consists of 20–100 diminutive and strikingly urn-shaped fertile leaves. These are sometimes in one continuous series, but their development is often interruped by the appearance of one or more series of larger sterile leaves. The spike may be simple or it may fork once or several times to form a dorsiventral, fanlike spray. The spike may persist and grow for several years. At any one time it may contain up to twenty-two living antheridia, from one of but a few cells to those holding ripe spermatozoids.

3. The development of the individual antheridium in this species resembles that in *Plagiochila asplenoides*, so far as this latter was followed by LEITGEB.

4. The nucleus of each of the spermatozoids, of which there are 25,000 or more in each antheridium, is at first globular, then pear-shaped, and finally becomes a slender cylinder or club. It has a peripheral, granular net or sometimes a series of transverse bands of chromatin. The young chromosomes, at early prophase of the last division, show series of minute component granules, the chromomeres, as do the chromosomes of the nuclei of the young spermatozoids organized immediately after this division.

5. No constant difference in form or size could be discovered among the chromosomes of the same or of different spermatozoids. Comparison of mitoses in male and female plants gave, likewise, no evidence of the presence of sex chromosomes here.

6. The blepharoplast first becomes clearly evident as a short rod when the nucleus of the young spermatozoid begins to elongate. It could not be constantly found at the poles of the last mitotic spindle. It was never seen in earlier mitoses. No clear case of vacuolization and fragmentation of the blepharoplast could be found.

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EXPLANATION OF PLATES I–III[3]

PLATE I

FIG. 5.—Horizontal section of apex of vegetative branch, showing initial cell, younger segments, and bases of leaves of two lateral ranks; above initial are transverse sections of trichomes arising from its ventral segments; ×130.

FIG. 6.—Approximately horizontal section of vegetative growing point, showing a branch rudiment at right; ×240.

FIG. 7.—Dorsal view of young leaf; ×240.

FIG. 8.—Approximately sagittal section of tip of young antheridial spike, seen from right side, showing apical cell and three young antheridia; ×240.

FIG. 9.—One-celled rudiment of antheridium with subtending leaf, in horizontal section of spike; ×240.

FIG. 10.—Part of approximately sagittal section of antheridial spike, show-

[3] All photographs and drawings made by the writer.

ing section of involucral leaf inclosing young antheridium, and basal end of next younger involucral leaf; ×240.

FIG. 11.—Part of approximately sagittal section of spike, showing young antheridium, with wall and spermatogenous cells defined, and inclosing involucral leaf; ×240.

FIG. 12.—Part of obliquely sagittal section of two successive involucral leaves, showing inclosed antheridia at about the level of antheridia 1 and 2 in fig. 36; antheridium at left is cut through adaxial half; ×240.

FIG. 13.[4]—Nearly transverse section of apex of young antheridial spike, showing stem initial, younger segments, form of involucral leaves and positions of inclosed antheridia; antheridia shown in dotted lines are at levels of spike below that of section from which rest of figure is drawn; ×130.

FIG. 14.—Part of approximately transverse section of sterile stem apex, showing initial with youngest ventral segment and trichome arising from next older ventral segment; ×160.

FIG. 15.—Adjoining section to that shown in fig. 14, showing base of trichome referred to; ×160.

FIG. 16.—Transverse section of antheridial spike below apex, showing stem, attachment of involucral leaves to latter, antheridial stalks in youngest involucres, and positions of older antheridia in outer involucres; ×30.

FIG. 17.—Transverse section near middle of mature antheridial spike, showing stem and completely closed basal portions of four successive involucral leaves, with transverse sections of two antheridial stalks; note absence of any broad ventral flap from basal portion of involucral leaf; ×30.

FIG. 18.—Transverse section of antheridial spike through two fullgrown antheridia, showing structure of stem and attachment of involucral leaves to latter; ×30.

FIG. 19.—Part of section similar to that shown in fig. 18, enlarged to show details, including thickening of outer cell walls of stem and involucre; ×130.

FIG. 20.—Transverse section of stem and single involucral leaf which, as occasionally happens, incloses two antheridia, the inner and upper being the younger; ×260.

FIG. 21.—Chloroplasts from leaves fixed in Flemming's solution; ×1340.

FIG. 22.—Horizontal section of young antheridium, showing stalk and upper surface of eight-celled body; ×240.

FIG. 23.—Lateral view of young antheridium, showing cells of abaxial surface; ×240.

FIG. 24.—Horizontal section of young antheridium, showing 2-rowed stalk, wall, and one of the two primary spermatogenous cells present at this stage; ×240.

FIG. 25.—Part of approximately horizontal section of spike, showing antheridium with four spermatogenous cells and stalk two cells wide; ×240.

[4] Figs. 13–19 and 25–27 are from slides prepared by Dr. B. H. GRAVE when he was a student at Johns Hopkins University.

FIG. 26.—Approximately sagittal section of antheridium from right side of spike, showing four of its eight spermatogenous cells; ×240.

FIG. 27.—Surface view of young antheridium from horizontal section of spike, showing arrangement of cells in wall; cf. fig. 28; ×250.

FIG. 28.—Surface view of young antheridium of *Plagiochila asplenoides*;[5] compare plan of arrangement of surface cells with that in fig. 27; ×250.

FIG. 29.—Lateral view of abaxial surface of young antheridium, showing arrangement of cells of wall; ×250.

FIG. 30.—Sagittal section of antheridium shown in fig. 29, showing wall and five of the ten spermatogenous cells; ×250.

FIG. 31.—Approximately sagittal section of older antheridium, showing positions of octant walls in spermatogenous group of cells; ×240.

PLATE II

FIG. 32.—Sagittal section of two-thirds grown antheridium, showing single row of stalk cells, wall, and spermatogenous cells (androgones); ×350.

FIG. 33.—*a*, sagittal section of fullgrown antheridium after formation of androcyte nuclei, showing over 1000 cells in longitudinal section; *b*, cross-section of stalk of fullgrown antheridium; ×220.

FIG. 34.—Lateral surface of mature antheridium, showing size and form of surface cells; from alcoholic specimen; ×125.

FIG. 35.—Lateral view of ruptured, living, ripe antheridium; ×125.

FIG. 36.—Horizontal section of forked antheridial spike, showing relative size of successive antheridia and position of involucral leaves; branch of spike (dotted lines) is from third section beyond that from which rest of figure is drawn. Two developing eggs (perhaps of tardigrade or nematode) lie beside stalk of antheridium no. 8; lowest involucral leaf incloses two antheridia; ×25.

FIG. 37.—Part of radial longitudinal section of axis of mature spike, showing character of thickened surface cells and thinner-walled axial cells of stem, also bases of antheridial stalk and involucre; ×170.

FIG. 38.—Transverse section of body of very young antheridium, showing median longitudinal wall which most frequently stands perpendicular to radius of stem passing through stalk as it does here; ×350.

FIGS. 39–42.—Transverse sections of body of antheridium, successive stages, showing sequence of cell walls which delimit spermatogenous cells from antheridial wall; arrow indicates direction of axis of spike from antheridium, and shows position of median wall in relation to sagittal plane; ×350.

FIG. 43.—View of top of young antheridium, showing positions of median and first quadrant walls; ×350.

FIG. 44.—Transverse section through base of antheridium, showing median wall and next following anticline in each half; ×350.

FIG. 45.—Transverse section of stalk of young antheridium; ×350.

FIG. 46.—Transverse section of stalk of ripe antheridium; ×350.

[5] Traced from LEITGEB, Vergleichende Untersuchungen. Heft II, Tav X, fig. 21.

FIG. 47.—Transverse section of two antheridia in same involucre; arrow indicates direction of axis; note that lower antheridium is further developed than upper (the next younger antheridium decidedly less developed than either of these); another involucre of this same spike also bore two antheridia; ×350.

FIG. 48.—Surface view of top of antheridium at age of that shown in fig. 32; ×350.

PLATE III

FIG. 49.—Transverse section of antheridium about age of that shown in fig. 32, showing four of the eight octants of spermatogenous cells; ×350.

FIG. 50.—Part of approximately sagittal section of antheridial spike, showing relative sizes and positions of eleven successive antheridia on one side of axis, to serve as index of ages of antheridia and spermatogenous cells shown in figs. 51–61; ×40.

FIG. 51.—Longitudinal section of very young antheridium (no. 1 of fig. 50) and its involucre; ×750.

FIG. 52.—Section (sagittal to spike) of antheridium no. 2 of fig. 50, showing stage of development and structure of nuclei; ×750.

FIG. 53.—Sagittal section of young antheridium (no. 3 of fig. 50); ×750.

FIG. 54.—Part of sagittal section of halfgrown antheridium, showing single wall cell and two spermatogenous cells (no. 4 of fig. 50); ×1400.

FIG. 55.—*a*, nucleus of androcyte reorganizing after last nuclear division (polar view) from antheridium no. 5 of fig. 50; ×1700. *b*, telophase from same antheridium but slightly more advanced than that in *a*, showing two sister androcyte nuclei (no. 5 of fig. 50); ×1400. *c*, later telophase of last nuclear division (no. 5 of fig. 50), showing chromatin net forming in androcyte nuclei; ×1700.

FIG. 56.—Young spermatozoids from antheridium no. 6 of fig. 50, showing nuclei slightly elongated (axis of last spindle in plane of page; that is, spermatozoids seen from edge; ×1400.

FIG. 57.—Similar view of two slightly older spermatozoids from antheridium no. 7 of fig. 50; ×1400.

FIG. 58.—*a*, spermatozoids from antheridium no. 8 of fig. 50 seen from edge; nuclei and blepharoplasts now somewhat elongated and bent; *b*, spermatozoid of same age and from same antheridium as those in *a*, seen from side of incipient coil instead of from edge; nucleolus no longer evident; chromatin in large granules; ×1400.

FIG. 59.—Still older spermatozoid from antheridium no. 9 of fig. 50 seen from edge, and showing greater elongation, with chromatin granules in transverse bands; ×1400.

FIG. 60.—*a*, two spermatozoids from antheridium no. 10 of fig. 50 seen from edge, showing but slight advance in development over that in fig. 59; *b*, transverse sections of two spermatozoids of same antheridium, showing peripheral position of chromatin in each band; ×1400.

FIG. 61.—Spermatozoid from antheridium no. 11 of fig. 50 seen partly from edge, showing slender spindle-like form; ×1400.

FIG. 62.—Spermatogenous cells from young antheridium (about age of no. 4 in fig. 50), showing organization of nucleus, with chromatin, nucleolus, and one or two other darkly staining bodies; ×1700.

FIG. 63.—Spermatogenous cell from young antheridium slightly older than that in fig. 54, showing organization of nucleus and cytoplasm; ×1700.

FIG. 64.—Young spermatozoid seen from edge (about age of those in fig. 57); ×1600.

FIG. 65.—Three young spermatozoids from antheridium no. 7 of slide 13: *a*, seen from edge of coil; *b*, *c*, seen more from the side, showing beginning of coiling; ×1600.

FIG. 66.—Two spermatozoids from antheridium no. 8 of slide 13, slightly older than that drawn in fig. 65, showing frequent arrangement of chromatin that gives spermatozoid nucleus the appearance of being spirally coiled; this spiral form not always distinguishable in spermatozoids between those of ages shown in figs. 65 and 67; ×1600.

FIG. 67.—Two pairs of spermatozoids from antheridium no. 9 of slide 13: *a* slight coiling; *b*, spindle-like form and aggregation of chromatin into bands; blepharoplasts not evident; ×1600.

FIG. 68.—Spermatozoid showing 1½ coils, from antheridium no. 10 of slide 13, with flagella(?) extending forward from tip of spermatozoid; ×1600.

FIG. 69.—Androcyte mother cell, showing chromatin net and distinct nucleolus (phase following that shown in fig. 54); ×1600.

FIG. 70.—Last androgone (androcyte mother cell), showing structure of nucleus just before initiation of last division (that which forms spermatozoid nuclei); ×1600.

FIG. 71.—Nucleus of androcyte mother cell showing late prophase of last mitosis; this and figs. 72–84, except 75, are from a single antheridium, and show range in stages of development that may occur in different parts of same antheridium); ×1600.

FIG. 72.—Prophase of last mitosis in antheridium, showing long rods and loops of rather deeply stained chromatin; ×1600.

FIG. 73.—Group of three adjacent nuclei, from same antheridium as that in fig. 71, showing elongated and bent chromosomes, and something of the transverse bandlike localization of chromatin granules; ×1600.

FIG. 74.—*a*, another nucleus from same antheridium as that from which figs. 71 and 72 were taken, showing more striking transverse banding of chromosomes; ×1600. *b*, two chromosomes of age shown in *a*, showing transverse banding in more detail; ×1750.

FIG. 75.—Nucleus (densely stained) between stages shown in figs. 74 and 76 (from different spike); ×1600.

FIG. 76.—Nucleus of androcyte mother cell in prophase, slightly more ad-

vanced than those in figs. 71–73, showing chromosomes more condensed but chromatin granules still peripheral in position; ×1600.

FIG. 77.—Two nuclei slightly more advanced than those shown in figs. 74 and 76; chromatin of the ten chromosomes more condensed and deeply stained; ×1700.

FIG. 78.—Late prophase of last mitosis in androcyte mother cell, showing chromatin thread broken to short thick chromosomes, with chromatin aggregated chiefly at surface; ×1600.

FIG. 79.—Still another nucleus of same antheridium as that used for fig. 78, showing contraction of chromosomes; most of chromatin still peripheral in position in each; ×1600.

FIG. 80.—Metaphase of last division in antheridium, showing chromosomes, spindle, and blepharoplast(?) at upper pole; ×1600.

FIG. 81.—Telophase of last mitosis in spermatogenous cell (androcyte mother cell), showing first step in organization of the two resulting spermatozoid nuclei; nucleus from same antheridium as that from which the last five figures were drawn; ×1600.

FIG. 82.—Metaphase of last division of androgone nucleus with blepharoplast (?) near upper pole; ×1600.

FIG. 83.—Late telophase of division of androcyte mother cell nucleus, showing chromatin granules in approximately ten groups at each pole, precise number of groups not determined; ×1400.

FIG. 84.—Polar view of one daughter nucleus of age shown in fig. 83; ×1400.

FIG. 85.—Polar view of nucleus of young spermatozoid of slightly later stage than those shown in figs. 81 and 83, although drawn from same antheridium with these and figs. 71–74 and 76–80, showing chromosomes with component granules (chromomeres); ×1700.

FIG. 86.—Two young spermatozoids showing triangular shape, in section parallel to last spindle; nuclei still nearly spherical, with chromatin grains distinct; ×1400.

FIG. 87.—Spermatozoid of next older antheridium of spike from which fig. 86 was drawn, showing slight elongation of nucleus and faint indication of longitudinal chains of chromatin granules; ×1600.

FIG. 88.—Young spermatozoid from antheridium next older than that in fig. 87, showing elongation of nucleus and blepharoplast and suggestion of transverse bands of chromatin; ×1300.

FIG. 89.—Two young spermatozoids, adjoining that figured in fig. 88, showing transverse sections of spermatozoid nuclei and peripheral position of chromatin granules; ×1600.

FIG. 90.—Spermatozoid from same antheridium as that used in fig. 88, showing slightly greater coiling, attenuation of anterior end of nucleus, and peripheral arrangement of chromatin granules; ×1600.

FIG. 91.—This and five following figures are from six successive antheridia

on same side of single spike; fig. 91 itself is of a very young spermatozoid from antheridium no. 1 of this spike, showing still globular nucleus with small and definite number of chromatin bodies; × 1300.

FIG. 92.—Two young spermatozoids from antheridium no. 3 of this spike, showing flattened sides and few large chromatin grains; ×1300.

FIG. 93.—*a*, *b*, Three young spermatozoids from antheridium no. 3 of this spike, showing blepharoplast beginning to elongate, while nucleus is still globular; ×1300.

FIG. 94.—*a-d*, Five spermatozoids from antheridium no. 4 of this spike: *a* from side, *b* in cross-section, *c* and *d* from edge; ×1300.

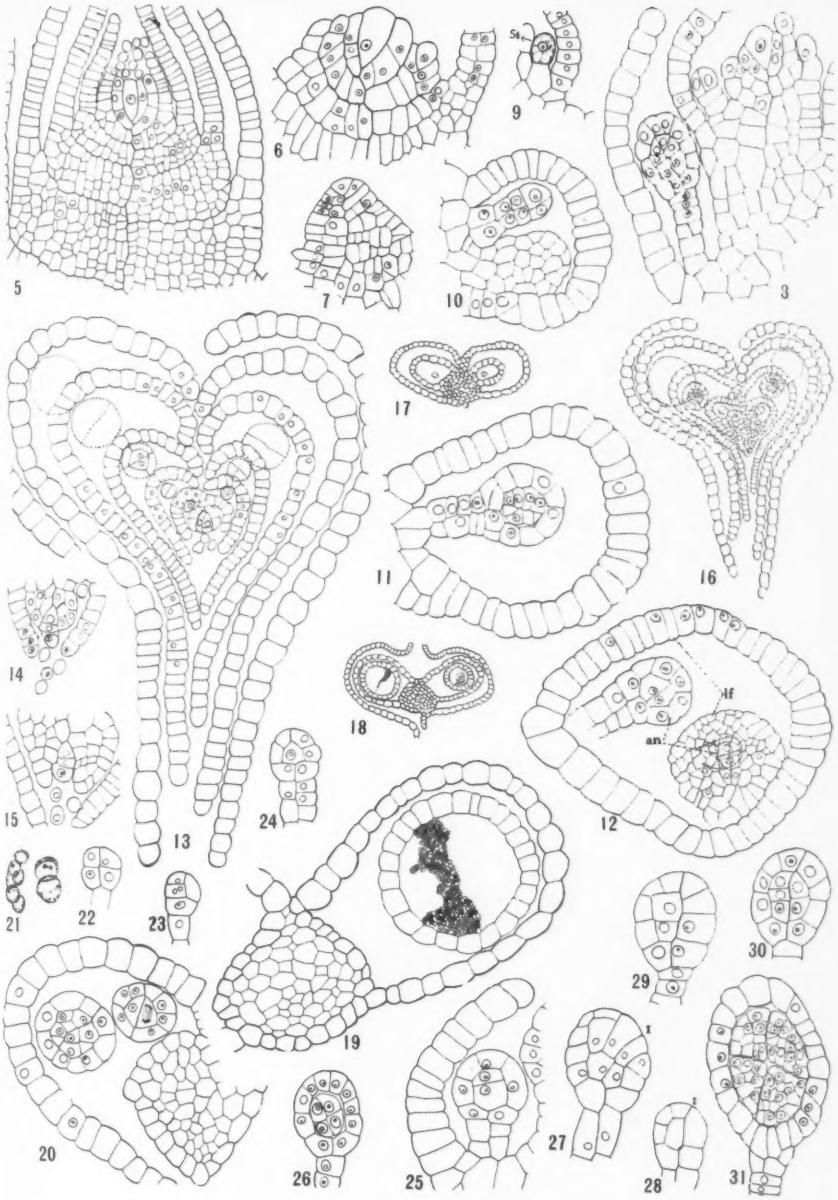
FIG. 95.—*a-d*, Four spermatozoids from different parts of one antheridium, no. 5 of this spike, showing further elongation and coiling, and peripheral position of chromatin. This latter often appears in five or six transverse bands (or turns of spiral) which in cross-section seem incomplete, as in same stage shown in fig. 60 *b*. In *c* the whole body of spermatozoid nucleus appears coiled nearly twice around like a corkscrew. This slide, rather faintly stained, showed no clear blepharoplast, cf. fig. 66; ×2600.

FIG. 96.—Spermatozoid from antheridium no. 6 of this series, showing marked coiling of nucleus; blepharoplast not evident; ×1300.

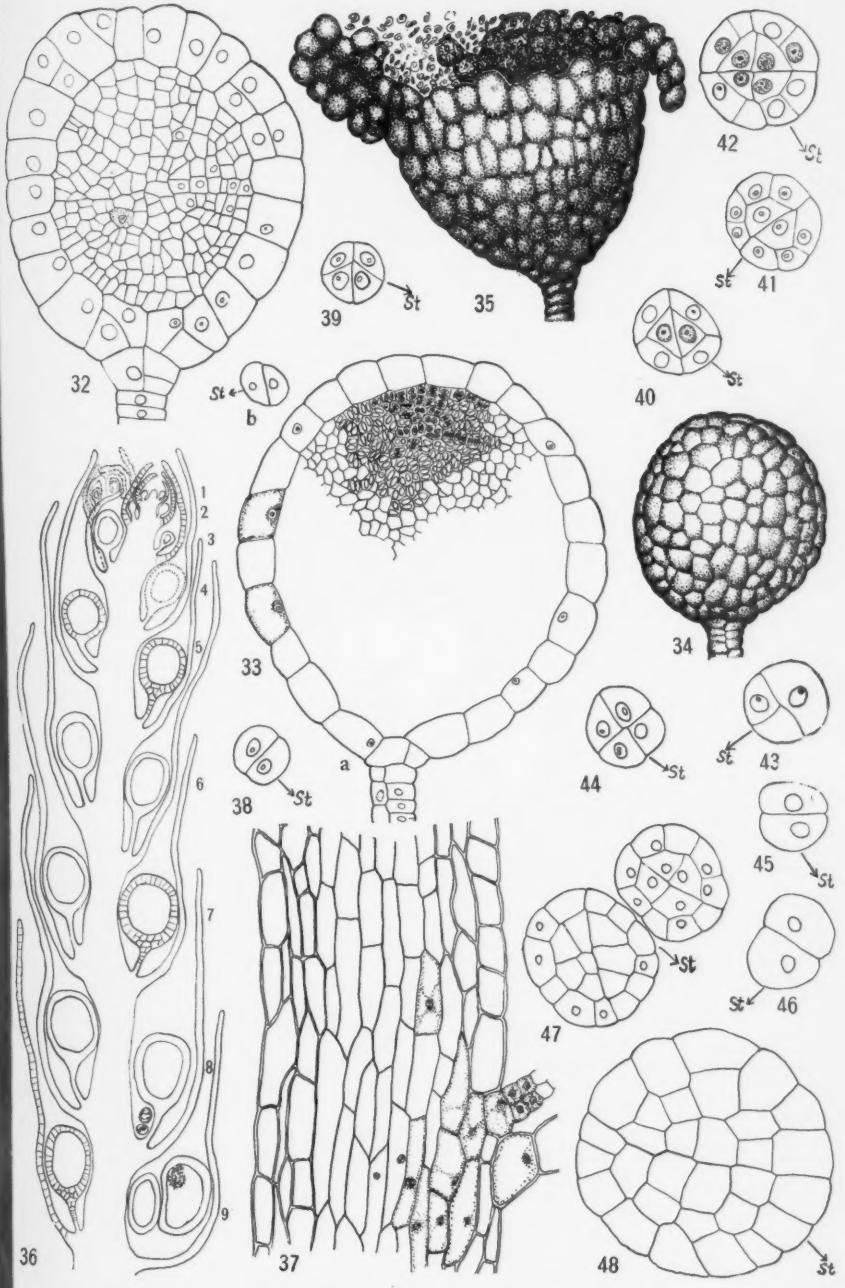
FIG. 97.—*a*, *b*, Three young spermatozoids from antheridium no. 7 of its spike, about age of that shown in fig. 94, showing distinct blepharoplast which is clearly free from nucleus; ×1300.

FIG. 98.—Nearly mature spermatozoid from antheridium no. 5 of same spike from which fig. 99 was drawn; ×1400.

FIG. 99.—Optical transverse sections of two coiled spermatozoids of same antheridium as those drawn in fig. 98, showing limosphere(?) and tubelike arrangement of chromatin granules; ×1700.



JOHNSON on PLAGIOCHILA



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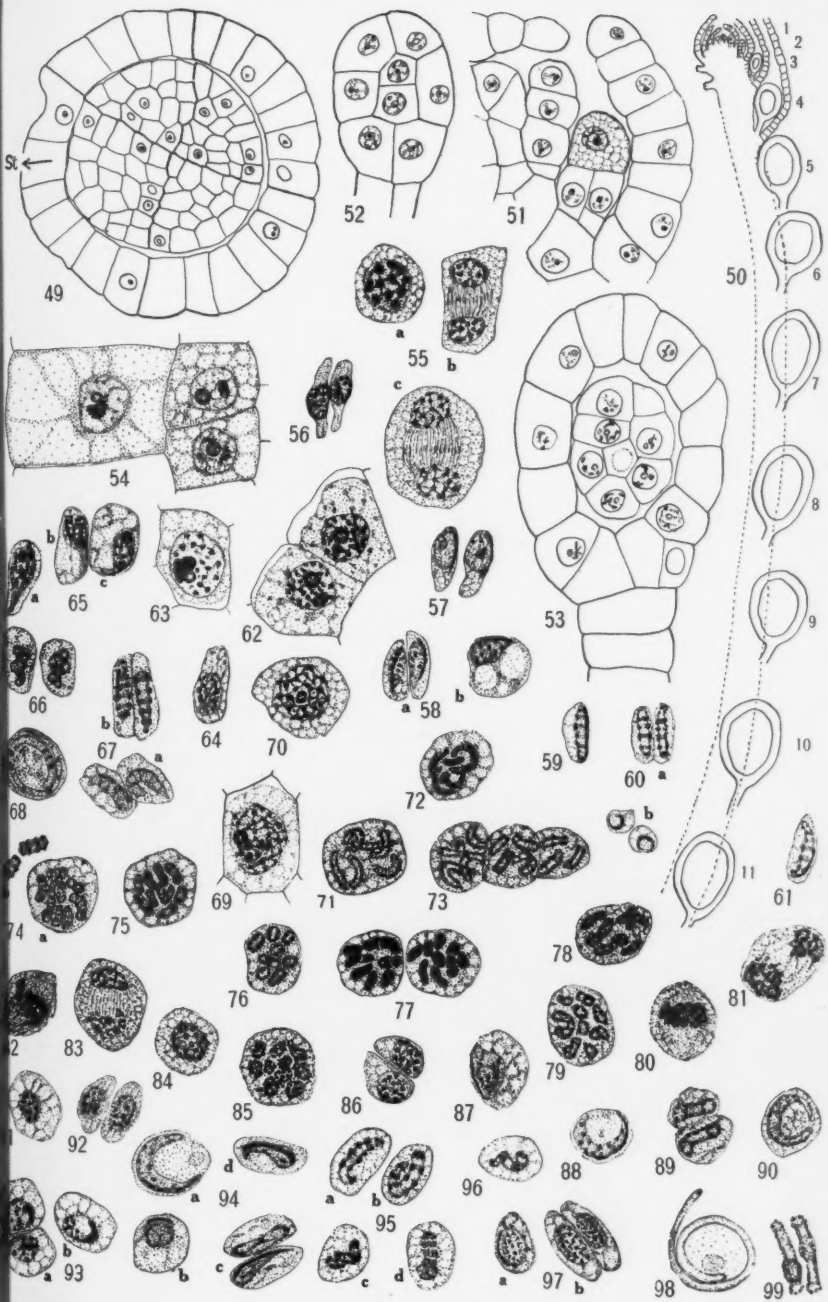

JOHNSON on PLAGIOCHILA

MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE[1]

II. PANICEAE AND ANDROPOGONEAE

GEORGE L. CHURCH

(WITH PLATES IV–VI)

In the first part of this study,[2] the importance of hybridization in the multiplication of species in the larger families and genera of plants, together with the substantiating cytological evidence, was reviewed. The cytology of the maturation of the pollen grains in thirteen species of grasses, representing the tribes Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae, was reported, with an account of the methods employed in the technique of the investigation. The species *Phalaris arundinacea* L. is a normal diploid; the other species are considered to have arisen by means of hybridization because of the evidence of polyploidy and abnormal cytological behavior.

In this article, the cytology of eighteen species and varieties representing the tribes Paniceae and Andropogoneae is described. Because of the notorious variability in the dichotomum type of *Panicum*, most of the investigation in the genus has been focused on this section.

Cytology of species

PANICEAE

Digitaria sanguinalis (L.) Scop., naturalized weed from Europe; pollen 30–40 per cent imperfect.—This species is tetraploid, showing 14 ringed bivalents at diakinesis. Cytomyxis, occurring at this stage, is believed to be the cause of the masses of chromatin often seen in the cytoplasm during the heterotypic division. Bivalent laggards are frequently seen in this first division, and may be extruded during the metaphase, which is very tardy in forming the plate. Homeotypic divisions are normal, but polycary is occasionally found.

Paspalum muhlenbergii Nash; pollen 30 per cent imperfect.—

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

[2] BOT. GAZ. 87: 608–629. 1929.

This species seems to be a diploid in the series in which 10 rather than 7 is the haploid number. The chromosomes here appear small and spherical as they do in *Panicum*. Whereas 10 bivalents usually appear at diakinesis, counts of 9 may be made definitely in many instances. Counts of metaphase plates of the heterotypic division show a similar aberration, even a few instances of 11 bivalents being noticed. Here again cytomyxis occurs to a considerable degree during the spireme stage, often persisting through diakinesis. Many of the latter stages may be found with chromatin disintegrating near large vacuoles in the peripheral cytoplasm of the mother cell. The heterotypic division is marked by many lagging bivalents in the metaphase, a large percentage of which are extruded. Anaphase laggards are less frequently left on the spindle at telophase. Cytoplasmic strands may be seen between mother cells at interkinesis; consequently the diad stage is seen presenting at times a polycaric appearance. The chromosomes are rather minute in the homeotypic division, but the telophase may be found with added extruded pieces of chromatin. Polycary, as it not infrequently occurs in the tetrad stage, is therefore to be expected.

PANICUM

DICHOTOMIFLORA

Panicum dichotomiflorum Michx.; pollen 20–30 per cent imperfect.—Counts of metaphase plates of the heterotypic division reveal 27 bivalent chromosomes, making this species hexaploid, reckoning 9 as the basic haploid number. Occasionally metaphases and anaphases of the first division present a considerable number of laggards, and extrusions on the spindle at the telophase are seen to follow as a result. The homeotypic divisions are quite regular, however, and very seldom does polycary occur. Cytomyxis is frequently observed at the spireme stage and at interkinesis.

CAPILLARIA

Panicum miliaceum L., cultivated in and advanced from the Old World; pollen 10–20 per cent imperfect.—Polar views of metaphase plates of the heterotypic division usually show 20 bivalents, although many definite counts of 18 have been made. This is doubtless a

tetraploid species originally having 10 as the basic haploid number, in common with such genera as *Paspalum*, *Sorghastrum*, and *Zea*. Occasional extrusions have been noted at the heterotypic metaphase together with lagging in the anaphase. Extranuclear chromatin may appear in the telophase as a consequence. The homeotypic metaphase often presents laggards, and polar views of regular spindles at times present only 18 chromosomes. The anaphase is quite regular, however. Cytomyxis has been observed at interkinesis, but no polycary has been found.

DICHANTHELIUM[3]

LANUGINOSA

Panicum lindheimeri Nash.—*P. huachucae*, *P. tennesseense*, and *P. implicatum* have for the most part been treated as varieties of the preceding species by FERNALD (11). Pollen is completely sterile in the species and its varieties.

Panicum lindheimeri Nash var. *typicum* Fern.—Diakinesis (fig. 44) usually reveals 9 bivalents, one or two pairs very loosely (if at all) united. Cytoplasmic chromatin may also be observed at this stage. Such extrusions are unquestionably due to the large amount of cytomyxis occurring at the spireme stage, and at times persisting through the diakinesis. Frequently whole anthers display cytomyxis to such an extent that it is doubtful whether any of the mother cells develop farther than the prophase. Lagging and extrusions are common phenomena throughout all the divisions. The early heterotypic anaphase (fig. 45) is marked by a very uneven separation of the bivalents, resulting in lagging in the later stage (fig. 46). Such laggards may persist as extrusions in the early diad stage (fig. 47). Figs. 50, 51, and 54 illustrate the striking irregularity of the homeotypic division. An occasional instance (fig. 53) may be found where apparently the homeotypic spindle does not form, but the chromatin is simply pulled into each half of the dividing cell, the split being initiated at the equatorial region in usual fashion. It is difficult to judge the degree to which such amitotically dividing cells may mature, since practically all of the pollen collapses. A difference in the num-

[3] Subgenus of which *P. dichotomum* is the type, cf. HITCHCOCK and CHASE 23. Examination in all cases is of the spring florets.

ber of chromosomes seems to exist in this species. Homeotypic equatorial plates (fig. 52) often show only 8 bivalents, while heterotypic plates (fig. 49) usually show 9. A loss of a bivalent as an extrusion could explain this discrepancy, yet counts of 8 have been made at diakinesis and followed throughout the succeeding division. Polycary is consistently displayed in the tetrads and not infrequently polyspory to the extent of a small additional pollen grain (fig. 55). Homeotypic telophases often display much extruded chromatin. Fig. 56 shows a typically shriveled mature grain.

Panicum lindheimeri Nash var. *septentrionale* Fern.—The same count of 9 bivalents is made clearly in diakinesis. This form revealed, however, much fewer irregularities than any of the dichotomum-like species of *Panicum* investigated. A few laggards were found at times in the heterotypic anaphase, and a slight degree of polycary was displayed in the tetrads. All other stages appeared quite normal.

Panicum lindheimeri Nash var. *fasciculatum* (Torr.) Fern.—Here again diakinesis shows 9 bivalents, many being very loosely paired. Cytomyxis in the prophase stages produces cytoplasmic chromatin that often persists through the diad stage. Lagging is common in the heterotypic divisions (figs. 57, 58). Instances of a collapsed condition of the heterotypic spindle are illustrated in figs. 59 and 60. Non-disjunction accompanied by lagging may give rise to a different assortment of chromosomes at the poles (fig. 60). Fig. 61 represents an unusually early occurring interkinesis (before completion of the diad split), in which a decided difference in the number of chromosomes in each nucleus is easily observed. The diad stage is commonly marked by extruded chromatin. Homeotypic divisions present lagging chromosomes only occasionally, but polycary is often found in the tetrads.

Panicum lindheimeri var. *implicatum* (Torr.) Fern.—In the degree to which the four forms of *P. lindheimeri* present irregularities, this variety ranks third, the varieties *typicum*, *fasciculatum*, and *septentrionale* ranking first, second, and fourth respectively. Cytomyxis in the prophase stages is observed only occasionally. Lagging and extrusions still characterize the majority of the heterotypic divisions however, and polycary is often found. The material furnished an abundance of examples of the heterotypic telophase displaying

laggards on the spindle obviously excluded from the nuclei. The homeotypic divisions, so far as observed, proceed rather normally.

Panicum subvillosum Ashe; pollen 70–80 per cent imperfect.—Nine is here the haploid number, but frequent deviations from this are encountered. Eight is a common count, 7 and 10 being occasional. Two or three pairs of chromosomes often are very loosely (if at all) joined in diakinesis, and they persist in this condition, usually as laggards, on the heterotypic spindle. Such univalents may be extruded as the spindle of the first division is forming; consequently, in making chromosome counts, great care was exercized to distinguish bivalents and univalents, and to determine the number in both polar and spindle views of both divisions. Cytomyxis is very common, even in the diakinesis, 3 or 4 cells being involved simultaneously and whole chromosomes passing between them. Laggards and extrusions abound in the reduction division. Late anaphases have been observed with 9 chromosomes at one pole and 7 at the other. Homeotypic divisions are typified by lagging only to a slighter degree. Polycary is common, the extra nuclei often assuming proportions of one-half to two-thirds the diameter of the normal nucleus of each member of the tetrad.

COLUMBIANA

Panicum tsugetorum Nash; pollen 90 per cent imperfect.—Nine holds fairly consistently as the haploid number of chromosomes. Conditions of irregularity differ only slightly in degree of occurrence from those mentioned in the preceding species, *P. subvillosum*. Metaphases of both divisions assemble very tardily. Extrusions occur for the most part in the first division, and are very evident in the diad stage and later in the tetrads (polycary).

SPHAEROCARPA

Panicum sphaerocarpon Ell.; pollen 80–90 per cent imperfect.—Typical cytological irregularities of the genus are illustrated in this species. Fig. 62 shows a diakinesis with 9 bivalents, several only loosely associated and probably a portion of one pair extruded. Fig. 63 shows the heterotypic metaphase with nearly half the complement of bivalents lagging at one pole of the spindle. Unequal numerical distribution of the disjoined mates is illustrated in the

anaphase (fig. 64). Extruded laggards and cytomyxis may be observed in the telophase (fig. 65). The homeotypic divisions (figs. 66, 68) again present laggards and extrusions from the heterotypic. Varying counts made of metaphase plates (fig. 67) may undoubtedly be attributed to the unequal segregation noticed in the first division. Polycary is frequently observed.

OLIGOSANTHIA

Panicum scribnerianum Nash; pollen 70–80 per cent imperfect.—An abundant amount of cytomyxis at diakinesis (fig. 69) is seen in this species. This illustration shows the stretched chromatin between the nuclei and the transference of whole chromosomes and even nucleoli to the cytoplasm of adjacent mother cells. This phenomenon may persist even up to the stage when the nucleolus has disappeared and the spindle is being initiated (fig. 70). Such a mother cell as the upper one of the two shown in fig. 70, with all of its chromatin gone, naturally disintegrates. Extruded chromatin, resulting from disruption of nuclei suffered during cytomyxis, persists in the metaphase of the first division (fig. 71). This figure also shows non-paired or loosely paired mates on the heterotypic spindle. The anaphase displays typical lagging (fig. 72). By the time the homeotypic division is reached, a decrease in the chromosome complement from the basic haploid number of 9 is often observed. This division is further marked by the extrusion of whole chromosomes and fragments (figs. 73, 74). As would be expected, polycary is common in the tetrads (fig. 75).

Echinochloa crus-galli (L.) Beauv., naturalized from Europe. This form corresponds closely to the variety *longiseta* of WIEGAND (**48**); pollen 10–20 per cent imperfect.—Diakinesis reveals 21 bivalents, the hexaploid complement. Cytomyxis is found in this stage and in the preceding spireme condition of the mother cells. Instances of non-paired chromosome mates may be seen on the heterotypic spindle. For the most part the divisions are quite normal, however, and consequently no polycary is found.

Echinochloa frumentacea (Roxb.) Link; introduced from the Orient as an escape from cultivation. HITCHCOCK (**22**) terms this species *Echinochloa crus-galli* (L.) Beauv. var. *edulis* Hitchc.; pollen 30–40 per cent imperfect.—Counts of metaphase plates of the heterotypic

division show the presence of 28 bivalents, the octoploid number. Examples of loose pairing and lagging may frequently be found in the heterotypic metaphase, but only occasionally do bivalents lag in the anaphase. Homeotypic divisions and tetrads are quite normal in appearance.

ANDROPOGONEAE

Miscanthus sinensis Anderss. var. *zebrinus* Beal, cultivated for ornament; pollen 40 per cent imperfect.—Heterotypic metaphase plates clearly show 21 bivalent chromosomes. Cytomyxis, although a common phenomenon at the spireme stage, is present to a remarkable degree in this species. Fully two-thirds of the spireme, including the nucleolus, may migrate to an adjacent mother cell. It is not uncommon to see the nucleoli of two cells fused in the midst of a migrating mass of chromatin. The phenomenon often persists through diakinesis and the beginning of the heterotypic division. An occasional case is found where the heterotypic spindle is initiated between two mother cells, obviously the result of cytomyxis in earlier stages. Occasional laggards may be observed in the first division, the mother cells often displaying large and numerous connections of cytoplasm with neighboring cells. It is not uncommon to find chromatin extruded in the telophase. Cytomyxis may be observed again in the telophase as well as at interkinesis. Lagging with accompanying extrusions is present to a similar degree in the homeotypic divisions. Polycary is rarely found, however, and is not very striking when it does occur.

Andropogon scoparius Michx.; pollen 20–30 per cent imperfect.—This species is octoploid, having the unusual complement of 21 bivalents and 14 univalents. The bivalents are easily counted in the diakinesis and at the heterotypic metaphase plate (fig. 77). The best estimation of the number of univalents is obtained in the spindle view of the metaphase of the first division (fig. 76), during which they are seen lagging in contrast to the bivalents at the plate. The size of the univalents is quite striking, being not more than half that of the univalents that undergo pairing. Many of these univalents never reach the plate, as is evidenced in the partly completed anaphase (fig. 78). Those univalents that reach the middle of the spindle are distributed at random to the poles, not dividing in the process. The diad stage may be observed with extrusions, chromatin at times

being stranded in the space between the separating halves of the mother cell. Bivalents may occasionally lag with the univalents in the homeotypic division (fig. 79), the latter pursuing the same course as in the first division. Polycary is quite common in the tetrads (fig. 80).

Andropogon furcatus Muhl.; pollen 10–20 per cent imperfect.—This species is decaploid, heterotypic metaphase plates showing 35 bivalents (fig. 83). Diakinesis displays a striking ring formation of the synaptic mates. Barring a sluggishness of chromosome action in the early stages of both metaphase and anaphase, the heterotypic division is quite regular (figs. 82, 84). Similar regularity is observed in the homeotypic division (fig. 85), and the tetrads have a normal appearance (fig. 86).

Sorghastrum nutans (L.) Nash; pollen 10–20 per cent imperfect.—Diakinesis and heterotypic metaphase plates clearly show 20 bivalents, the tetraploid number. Occasionally a laggard may be seen in the metaphase of the first division, but regularity is the rule. Not only does the bivalent nature of the chromosomes reveal itself at this stage, but often the homeotypic split is manifested in an incipient condition, the whole presenting a linear tetrad appearance. Anaphases frequently display considerable irregularity in the early stage, but the late condition is quite normal. Cytomyxis, present in the prophase, may be observed occasionally at interkinesis. The homeotypic division is regular in appearance, as are the tetrads.

Discussion

Polyploidy

In the preceding article, the phenomenon of polyploidy was discussed as an important criterion of hybridization. Of special interest in the present reported series are the hexaploids *Panicum dichotomiflorum*, *Echinochloa crus-galli*, and *Miscanthus sinensis* var. *zebrinus;* the octoploids *Echinochloa frumentacea* and *Andropogon scoparius;* and the decaploid *Andropogon furcatus*.

Dysploidy

A further numerical comparison of the chromosome complements of the species of grasses investigated reveals some instances where

the haploid number is not a definite multiple of the basic number for the group. For example, in *Phalaris* the usual series of 7 holds for *P. arundinacea* and its tetraploid variety *picta;* yet *P. canariensis* has a haploid number of only 6. Again, in *Panicum* a new series of 9 appears to be established. Irregular deviations from even this factor are found, however. In *P. lindheimeri* var. *typicum* and in the variety *fasciculatum*, counts of 8 instead of 9 are frequently obtained. In *P. subvillosum*, not only counts of 8, but counts of 7 and 10 may be instanced. Although *P. dichotomiflorum* seems to hold to the 9 series with 27 bivalents, *P. miliaceum* only occasionally shows 18 bivalents, 20 being the more established haploid complement. Table I shows that 10 is a well established number in *Paspalum*, yet counts of 9 and of 11 may be obtained in *P. muhlenbergii*. Such irregular deviations from the fundamental haploid base in a polyploid series have been conveniently termed dysploids (JEFFREY **30**).

Previous investigation in various genera of grasses has displayed similar cases of dysploidy. The new haploid number of 12 has been reported in *Oryza sativa* (ISHIKAWA **27**), yet counts of 10 to 14 have been made in several so-called "race mutants" of this species (NAKATOMI in AASE and POWERS **1**). Races of *Hordeum* (KIHARA **32**) and *Secale* (GOTOH **19**) with 8 as the haploid number instead of the usual 7 have been reported. Deviations from the haploid count of 10 are found in *Euchlaena mexicana* (LONGLEY **35**) and certain races of *Zea mays* (KUWADA **34**). Segregates of *Avena sativa* show 40, 41, or 44 instead of the usual somatic complement of 42 (HUSKINS **26**). A considerable amount of dysploidy is seen in various species and hybrids in *Saccharum* (BREMER **3, 4**). The obvious hybrid origin of many of these dysploids is particularly significant.

Outside of the Gramineae, hybridity and dysploidy may again be frequently correlated. *Oenothera* "mutants" are found with 15 and 16 chromosomes instead of the usual 14 (LUTZ **36**). Very irregular series are found in the much inter-crossed species of *Crepis* (ROSENBERG **41**) and *Lactuca* (ISHIKAWA **28**).

ABSENCE OF POLYPLOIDY.—Before leaving the general subject of polyploidy and its relation to hybridization, the converse situation must be considered; namely, the absence of chromosome multiplication where other obvious evidence of crossing is present. It has been

noted that in the 8 forms of the *dichotomum*-like species of *Panicum* studied there is no multiple deviation from the haploid number of 9; yet these forms bear just as obviously the characteristics of hybrids

TABLE I

EXTENT OF POLYPLOIDY IN THE GRAMINEAE

	DIPLOID	TETRAPLOID	HEXAPLOID	OCTOPLOID	DECAPLOID
Digitaria sanguinalis*		14			
Paspalum stoloniferum (Marchal 37)	10				
Paspalum dilatum (Marchal 37)		20			
Paspalum muhlenbergii*	10				
Panicum dichotomiflorum*			27		
Panicum miliaceum*		20			
Panicum lindheimeri* (4 vars.)	9				
Panicum subvillosum*	9				
Panicum tsugetorum*	9				
Panicum sphaerocarpon*	9				
Panicum scribnerianum*	9				
Echinochloa crus-galli*			21		
Echinochloa frumentacea*				28	
Imperata arundinacea (Bremer 4)	10				
Miscanthus sinensis v. zebrinus*			21		
Ischaemum timorense (Bremer 4)	10				
Saccharum (Bremer 4)			30	40	
Erianthus (Bremer 4)			30		
Andropogon scoparius*				21 bivalents + 14 univalents	
Andropogon furcatus*					35
Cymbopogon (Kuwada 33)	10				
Sorghum (cf. Ishikawa 27)	10				
Sorghastrum nutans*		20			
Tripsacum (Longley 35)			30		
Euchlaena (Longley 35)	10	20			
Coix (Longley 35)	10				
Zea (cf. Gaiser 13)	10				

* Species investigated in this research. Numbers refer to the haploid complement.

in their closely intergrading external characters, sterile pollen, polycary, and lagging chromosomes.

While perhaps a satisfactory explanation of such a case is lacking, it is not without parallel instances. The most striking case among the Gramineae is that of the 37 varieties of *Hordeum vulgare* all showing a haploid number of 7 (EMME **9**). The same count holds for the 10 other species of the genus investigated (GAISER **13**). Many of the species and varieties of *Aesculus* are bad hybrids (HOAR **24**), yet, except in one instance, they are all diploids. Similar situations may be cited in *Wisteria* (ROSCOE **38**) and *Anthurium* (GAISER **14**).

LAGGING UNIVALENTS.—In the previous article, the presence of 14 univalents in the hexaploid *Spartina alterniflora* var. *glabra* was compared with similar conditions in several well known natural and experimental hybrids.

The presence of univalents in octoploid types of a regular polyploid series, such as is found in *Andropogon scoparius* (with 21 bivalents and 14 univalents) is apparently rare. Analogies appear, however, in connection with high multiples in dysploid series of hybrids. Among these are the varieties of *Saccharum officinarum*. The type species shows regularly 40 bivalents, but in "Black Cheribon" for example, 36 bivalents and 8 univalents appear (BREMER **3**). High chromosome numbers involving lagging univalents are seen again in sterile fern hybrids, as *Polypodium schneideri* (FARMER and DIGBY **10**) and *Nephrolepis exaltata* var. *bostoniensis* (JEFFREY and HICKS **31**).

NON-PAIRING, LAGGING AND EXTRUSION.—In the Festuceae, instances have been noted where unpaired chromosome mates in diakinesis are seen to lag on the spindle of the first division, and later become extruded into the cytoplasm. *Echinochloa crus-galli* and *E. frumentacea* both display similar laggards on the heterotypic spindle. In *Miscanthus sinensis* var. *zebrinus* such univalents never reach the plate during the first division, but remain at the poles. *Panicum lindheimeri* var. *typicum* and *P. sphaerocarpon* both show a considerable amount of non-pairing at diakinesis. Again, it was noted in *P. subvillosum*, *P. tsugetorum*, and *P. scribnerianum* that loosely associated bivalents often lag in the heterotypic metaphase. These conditions, together with those of lagging and extrusion of bivalents

in both maturation divisions, such as are found in *Paspalum muhlenbergii* and several species of *Panicum*, have been correlated previously with the same conditions in known or suspected hybrids.

NON-DISJUNCTION.—In the case of *Panicum lindheimeri* var. *fasciculatum*, a definite case of non-disjunction has been demonstrated in the heterotypic division (figs. 59, 60). This is the particular type of unequal chromosome distribution in which one or two bivalents may approach and nearly reach the equatorial plate, but are pulled undivided to one of the poles. Instead of the usual 9/9 segregation in the anaphase, counts of 10/8 or 11/7 (fig. 61) are consequently made, with a resultant difference in the chromosome complements of the pollen grains if they mature.

Non-disjunction has been reported frequently in hybrids. *Oenothera lamarkiana* may give anaphase segregations of 8/6 or 9/5 instead of the usual 7/7 (SINOTO **44**). In the tetraploid species of *Datura*, segregates of 23/25 instead of 24/24 are quite regularly found (BELLING and BLAKESLEE **2**). The same situation has been recently reported in *Nicotiana alata* var. *grandiflora*, where 8/10 may often be the count in the anaphase instead of 9/9 (RUTTLE **42**).

When gametes of different numerical chromosome equipments mature as a result of unequal distribution or non-disjunction, it is obvious that they are functional in producing dysploid offspring, the many instances of which have been cited in a discussion of that particular type of polyploidy. Such is the opinion of HEILBORN (**21**) regarding the striking dysploid series in *Carex*. SHARP (**43**) states:

> Nothing is more reassuring in cytology at the present time than what we are able to ascertain concerning the rôle of chromosomes from their occasional misbehavior. In such phenomena as non-disjunction and polyploidy, nature performs almost before our eyes a series of experiments from which we should be dull indeed if we were to learn nothing..... We have almost everything to learn about the causes of such aberrations, but it is already clear that they play a part in the production of new races; and when we consider the multiploid series of species in many genera and families we can have little doubt that they have functioned in the origin of species also.

POLYSPORY.—In an investigation of 31 species and varieties of grasses, sterile pollen has been correlated repeatedly with polycary. It is noteworthy, however, that a representative from the

much hybridized group of *Panicum*, *P. lindheimeri* var. *typicum*, presents apparently the only case of polyspory in the Gramineae yet reported.

CYTOMYXIS.—The phenomenon of cytomyxis has been noted often in the cytological descriptions of the grasses in this study. While the pollen mother cells are in the spireme condition, considerable quantities of chromatin may be ejected from the nucleus and incorporated into the cytoplasm of adjacent mother cells. Anthers displaying more advanced stages show many mother cells with cytoplasmic chromatin, and adjacent shrunken remains of cells that have given up the greater portion of their nuclear contents, clearly demonstrating the result of excessive ejection of spireme chromatin. This type of chromatin loss has been termed cytomyxis (GATES **16**).

During the course of spireme cytomyxis, cytoplasmic strands may be formed in varying degrees of number and size between the mother cells. These intercommunicating strands often persist until the homeotypic division is well under way. They usually disappear, however, by the time the tetrads have formed. Chromatin or chromosomes stranded between cells in diakinesis, heterotypic metaphase, or interkinesis may or may not be assisted in migration by cytoplasmic bridges.

The first type of the grasses displaying cytomyxis are those in which the phenomenon is seen only in the spireme stage, but with resultant extrusions persisting in later stages. These are *Andropogon scoparius*, *Digitaria sanguinalis*, *Spartina michauxiana*, and *Festuca rubra*.

A greater number of the species investigated show such chromatin loss to the extent that the process is still manifested in diakinesis. Among these have been noted *Ammophila breviligulata*, *Alopecurus pratensis*, *Phalaris canariensis*, *Paspalum muhlenbergii*, and *Echinochloa crus-galli*. Cytomyxis has been observed in abundance at diakinesis in the dichotomum type of *Panicum*. Whereas all of these species are very sterile, it is interesting to note that the greatest amount of cytomyxis is correlated with the greatest amount of irregularities in the maturation divisions. A series may thus be arranged to show: a first group of *P. lindheimeri* var. *typicum*, *P. subvillosum*,

and *P. scribnerianum;* a second group of *P. lindheimeri* var. *fasciculatum* and *P. tsugetorum;* and a third group of *P. lindheimeri* var. *implicatum* and *P. sphaerocarpon.*

An excessive amount of cytomyxis in all stages of the prophase may result in irregularities in the heterotypic metaphase in which chromosomes on or off the spindle may be partly stranded in adjacent cells. This condition has been noted in *Alopecurus geniculatus* var. *aristulatus* (occasionally in *A. pratensis*), *Spartina alterniflora* var. *glabra*, and *Phalaris arundinacea* var. *picta*. In the case of *Miscanthus sinensis* var. *zebrinus*, the heterotypic spindle has been seen stranded between two mother cells.

Similar phenomena of chromatin transference at times are observed during interkinesis. Among such cases are *Miscanthus*, where cytomyxis has been observed in all stages, and the following where it has been observed previously in prophase: *Festuca duriuscula*, *Panicum dichotomiflorum*, *P. miliaceum*, and *Sorghastrum nutans*.

Cytomyxis has often been reported in pollen mother cells, although naturally in the light of various interpretations. GREGORY (20) noted the exchange of spireme chromatin in a sterile race of *Lathyrus odoratus*, and thought it to be some peculiar type of cell division. ROSENBERG (40) noted spireme cytomyxis in *Drosera longifolia* and SINOTO (45) in *Iris japonica*. Both of these investigators believe the phenomenon to be an artifact due to faulty fixation.

DIGBY (5) noted the ejection of chromatin during the spireme stage in *Galtonia candicans*. Inasmuch as nucleolar chromatin is believed to be involved, the ejections are termed nucleolar buds. They do not persist after the prophase. Figures published later (DIGBY 6) concerning the reduction division of this *Galtonia* seem to indicate obvious hybrid characteristics. "Chromatic" or "nucleolar droplets" of the same origin have been reported in *Polypodium schneideri* (FARMER and DIGBY 10), *Primula kewensis* (DIGBY 7), and *Crepis virens* (DIGBY 8), concomitant hybrid divisions being noted in all instances. That cytomyxis can be responsible for the degeneration of pollen mother cells and even whole anther sacs has been recognized, although strangely enough the investigators cite such conditions as normal. This is the opinion of FRASER (12) reporting on *Vicia faba*, and of WEST and LECHMERE (47) on *Lilium candidum*.

Cytomyxis recognized as an abnormality has been reported in

several definitely known hybrids. At the spireme stage it has been seen in *Oenothera rubrinervis* (GATES 15) and *O. gigas* (GATES 16). A hybrid form of *Typha angustifolia* shows the phenomenon at diakinesis (ROSCOE 39). Instances of its further appearance at interkinesis are found in *Lactuca sativa* (GATES and REES 18) and *Lathrea squamaria* (GATES 17).

It will be noted in a comparison between the grasses in this research and the cases of cytomyxis just cited, that the present study reveals a wider extent of the abnormality throughout the stages of the maturation divisions. Cytomyxis at the spireme stage is rather common, but it has been reported but once in diakinesis and apparently not at all in the heterotypic division such as is described in *Alopecurus*, *Phalaris*, and *Spartina*. The case of *Miscanthus* showing a spindle stranded between two mother cells seems to be thus far unique; yet, in all of these cases the cytoplasm of the cells involved shows absolutely no shrinkage. An occasional instance of an anther that has not been infiltrated with nitrocellulose, due to a failure in pricking, displays much distorted cytoplasm in the mother cells but scarcely any dislodgment of the chromatin, and certainly no ejection into neighboring cells, particularly if the anther in question happens to have reached the diakinesis or a later stage. Furthermore, the employment of such a rapid fixative as Carnoy's fluid together with an exhaust pump has not produced shrinkage in anthers displaying more normal conditions in the same material in question; hence it would seem that the phenomenon of cytomyxis cannot be an artifact. On the other hand, the occurrence of this abnormality in connection with hybrids is very striking. In contrast, the examination of considerable material in the case of *Phalaris arundinacea*, a normal diploid, has revealed no cytomyxis. Finally, even if the phenomenon can be proved not to be an exclusively hybrid characteristic, its occurrence is manifestly increased in obvious hybrids such as are described in *Panicum*.

Conclusions

PANICEAE

Digitaria sanguinalis.—This very common weed is representative of a widespread genus of some 60 species, 12 occurring in the southeastern United States. The species here considered is listed as being

very variable in GRAY'S manual, yet so far as the material investigated reveals, this is another "settled down" tetraploid species like *Dactylis glomerata* and *Spartina michauxiana*. In these forms the outer evidence points to hybridity, yet cytologically such evidence is concealed. Except for the presence of some bad pollen, these cases suggest those termed crypthybrids by JEFFREY (**29**).

Paspalum muhlenbergii.—This genus has about the distribution of *Digitaria*, with some 200 species, 50 of which are in the United States. The one here considered shows hybrid tendencies in lagging chromosomes, sterile pollen, and frequent irregularities in chromosome count.

Panicum.—This huge widespread genus of 500 species, with about 150 in the United States, presents a promising group for the appearance of hybrids, as is shown in the following subgenera:

Dichotomiflora.—The hexaploid *P. dichotomiflorum*, displaying irregularities in the reduction division and sterile pollen, must have a hybrid ancestry. It is noteworthy that transitional varieties to the next section have been found (SVENSON **46**).

Capillaria.—*P. miliaceum* seems to be a fairly settled species, yet occasional lagging chromosomes, bad pollen, and differences in the chromosome count indicate an uncertain purity of origin.

Dichanthelium.—Representatives of four tribes in this subgenus all show evidence pointing to a considerable degree of hybridization between the species. Although polyploidy is absent, there is presented extreme variability, very sterile pollen, much lagging in both maturation divisions, irregularities in chromosome count, cytomyxis, polycary, and even polyspory in one instance, the last phenomenon being apparently rare in the Gramineae.

Echinochloa.—This is not a particularly large genus, there being known only 10 species, with 4 in the United States. A rather high degree of polyploidy is reached, with the hexaploid number in *E. crus-galli* and the octoploid in *E. frumentacea*.

ANDROPOGONEAE

Miscanthus sinensis var. *zebrinus*.—The apparent hexaploid count of 21 bivalents seems to be anomalous in this species, since it deviates from the usual 10 series of related genera, particularly *Sac-*

charum. Deviations are not unknown, however, even in this latter genus (GAISER 13). Lagging chromosomes, much cytomyxis, as well as sterile pollen point to an evident hybrid ancestry for this variety. BREMER (4) has investigated several species in closely related genera of this tropical group, and considers many of them hybrids on the basis of polyploidy alone.

Andropogon.—Here again we are dealing with a large tropical genus of about 150 species, some 30 of which are found mostly in the southeastern United States. Certainly if polyploidy, even of itself, is a criterion of hybridity, it is here very strikingly instanced in the octoploid *A. scoparius* and the decaploid *A. furcatus*. *A. scoparius* has been noted in several varieties in this region (HUBBARD 25), and the presence of univalent chromosomes, polycary, and sterile pollen makes the evidence overwhelming for its hybrid origin. *A. furcatus* is equally outstanding in its regularity of reduction divisions and only very small amount of sterile pollen. Its hybrid origin may be more remote than that of *A. scoparius*, but its decaploidy attests such a lineage as has been previously stated.

Sorghastrum nutans.—This species is representative of a genus of 10 species, 3 of which occur in the United States, east of the Rockies. The evidences for hybridization as a factor in its origin (tetraploidy and a slight amount of chromosome irregularity and pollen sterility) are not striking, but point to the possibility nevertheless.

Summary

1. The following representatives of the tribes Paniceae and Andropogoneae have been investigated:

DIPLOIDS.—*Paspalum muhlenbergii*, *Panicum lindheimeri* in its four varieties, *P. subvillosum*, *P. tsugetorum*, *P. sphaerocarpon*, *P. scribnerianum*.

TETRAPLOIDS.—*Digitaria sanguinalis*, *Panicum miliaceum*, *Sorghastrum nutans*.

HEXAPLOIDS.—*Panicum dichotomiflorum*, *Echinochloa crus-galli*, *Miscanthus sinensis* var. *zebrinus*.

OCTOPLOIDS.—*Echinochloa frumentacea*, *Andropogon scoparius*.

DECAPLOID.—*Andropogon furcatus*.

2. Lagging univalents are found in *Andropogon scoparius*.

3. Non-disjunction is reported in *Panicum lindheimeri* var. *fasciculatum*.

4. Polyspory is found only in *Panicum lindheimeri* var. *typicum*.

5. Irregularities in the chromosome count are reported in several members of *Panicum*.

6. Cytomyxis is found to extend throughout the diakinesis and heterotypic metaphase in several species.

7. Varying degrees and combinations of non-pairing, lagging and extrusion, cytomyxis, polycary, and sterile pollen are found in the species investigated.

8. Polyploidy or cytological abnormalities of the maturation divisions or both are considered as evidence of the hybrid origin of these species.

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EXPLANATION OF PLATES IV–VI

PLATE IV

×2540 diameters

Panicum lindheimeri Nash var. *typicum* Fern.

FIG. 44.—Diakinesis showing 9 bivalents, one loosely paired; note cytoplasmic chromatin.

FIG. 45.—Early heterotypic anaphase, some bivalents late in splitting.

FIG. 46.—Heterotypic anaphase.

FIG. 47.—Heterotypic telophase; chromatin in path of obliterated spindle.

FIG. 48.—Somatic division (cell from young ovule tissue) contrasting with reduction division.

FIG. 49.—Heterotypic metaphase, polar view, showing 9 bivalents.

FIG. 50.—Homeotypic metaphase showing laggards.

FIG. 51.—Homeotypic anaphase showing laggards.

FIG. 52.—Homeotypic metaphase, polar view, revealing only 8 chromosomes.

FIG. 53.—Homeotypic anaphase, irregular division.

FIG. 54.—Homeotypic anaphase.

FIG. 55.—Tetrad showing polycary and polyspory.

FIG. 56.—Abortive pollen grain.

Panicum lindheimeri Nash var. *fasciculatum* (Torr.) Fern.

FIG. 57.—Heterotypic metaphase showing laggards.

FIG. 58.—Heterotypic anaphase showing laggards.

FIG. 59.—Heterotypic anaphase; note collapsed spindle.

FIG. 60.—Same; note non-disjoined bivalents passing to one pole.

FIG. 61.—Heterotypic telophase; note different number of chromosomes in nuclei.

PLATE V

×2540 diameters

Panicum sphaerocarpon Ell.

FIG. 62.—Diakinesis showing 9 bivalents, several loosely paired.

FIG. 63.—Heterotypic metaphase; note laggards.

FIG. 64.—Heterotypic anaphase.

FIG. 65.—Heterotypic telophase; note chromatin extruded from nuclei and cytomyxis.

FIG. 66.—Homeotypic metaphase; note extrusions and laggards.

FIG. 67.—Two polar views of homeotypic metaphases showing 8 and 10 counts.

FIG. 68.—Homeotypic anaphase.

Panicum scribnerianum Nash

FIG. 69.—Diakinesis in 3 mother cells showing cytomyxis.

FIG. 70.—Diakinesis in 2 mother cells, lower having nearly 2 chromosome sets as result of cytomyxis.

FIG. 71.—Early heterotypic metaphase; note extrusions.

FIG. 72.—Heterotypic anaphase; note bivalent laggards.

FIG. 73.—Homeotypic metaphase; note laggards and extrusions.

FIG. 74.—Homeotypic telophase; note extrusions.

FIG. 75.—Tetrad showing polycary.

PLATE VI

×2300 diameters

Andropogon scoparius Michx.

FIG. 76.—Heterotypic metaphase showing bivalents at plate and 14 univalent laggards on spindle.

FIG. 77.—Heterotypic metaphase, polar view, showing 21 bivalents and 12 univalents.

FIG. 78.—Heterotypic anaphase; some univalents still at poles.

FIG. 79.—Homeotypic divisions; both bivalent and univalent laggards in metaphase.

FIG. 80.—Tetrad showing polycary.

FIG. 81.—Somatic anaphase (cell from young ovule tissue); note regularity. *Andropogon furcatus* Muhl.

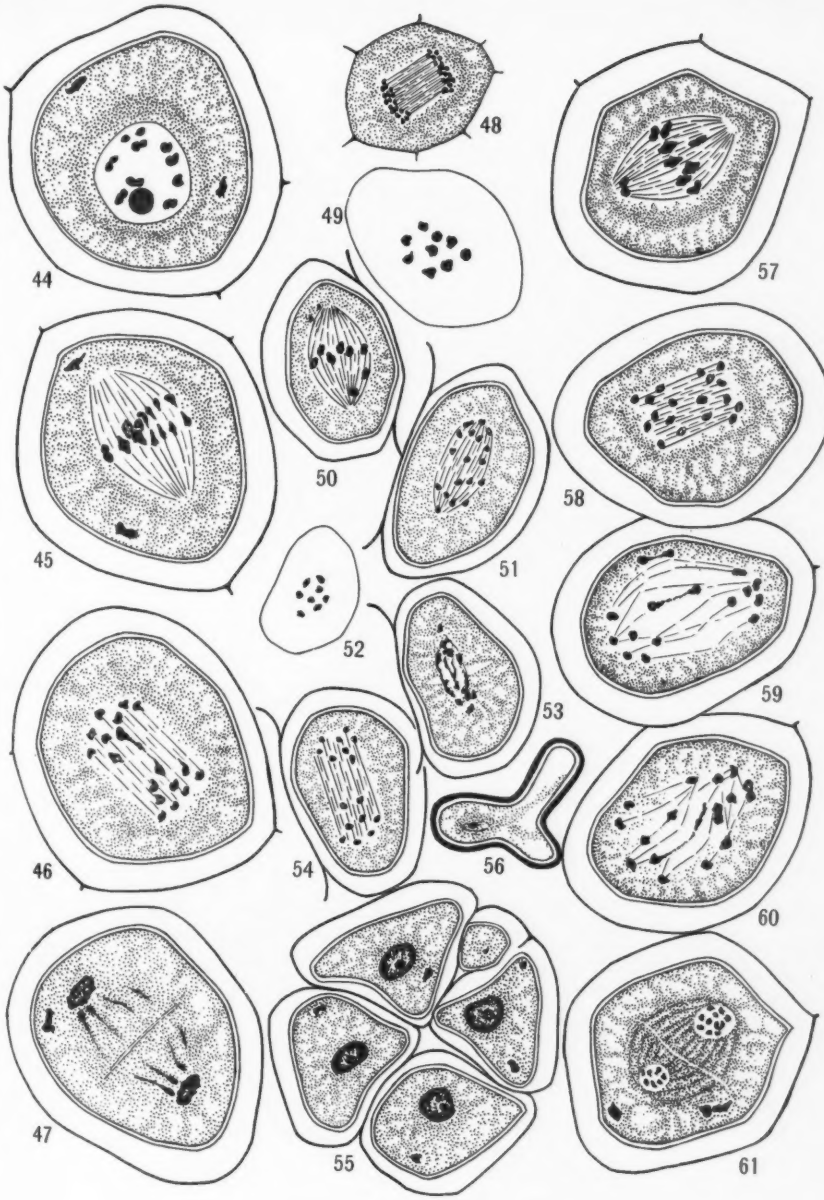
FIG. 82.—Heterotypic metaphase.

FIG. 83.—Heterotypic metaphase, polar view, showing 35 bivalents.

FIG. 84.—Heterotypic anaphase.

FIG. 85.—Homeotypic divisions.

FIG. 86.—Tetrad presenting normal condition in contrast to fig. 80.



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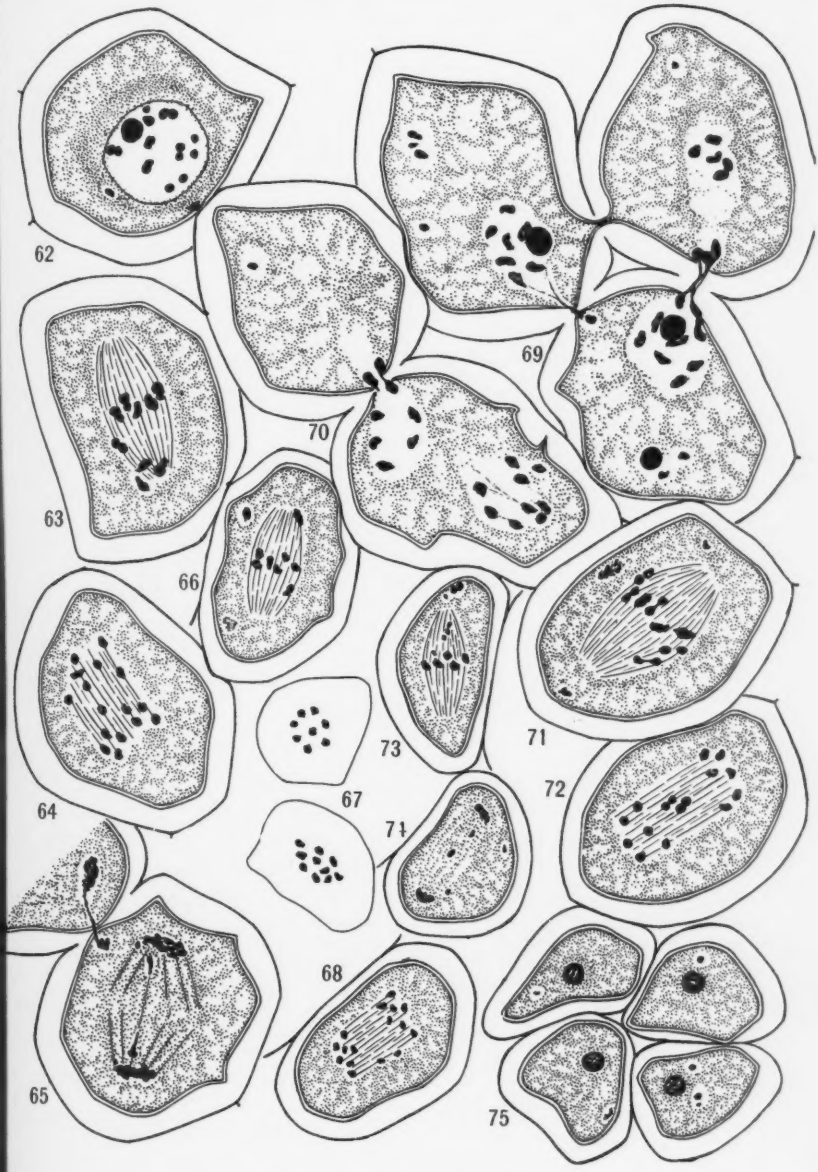

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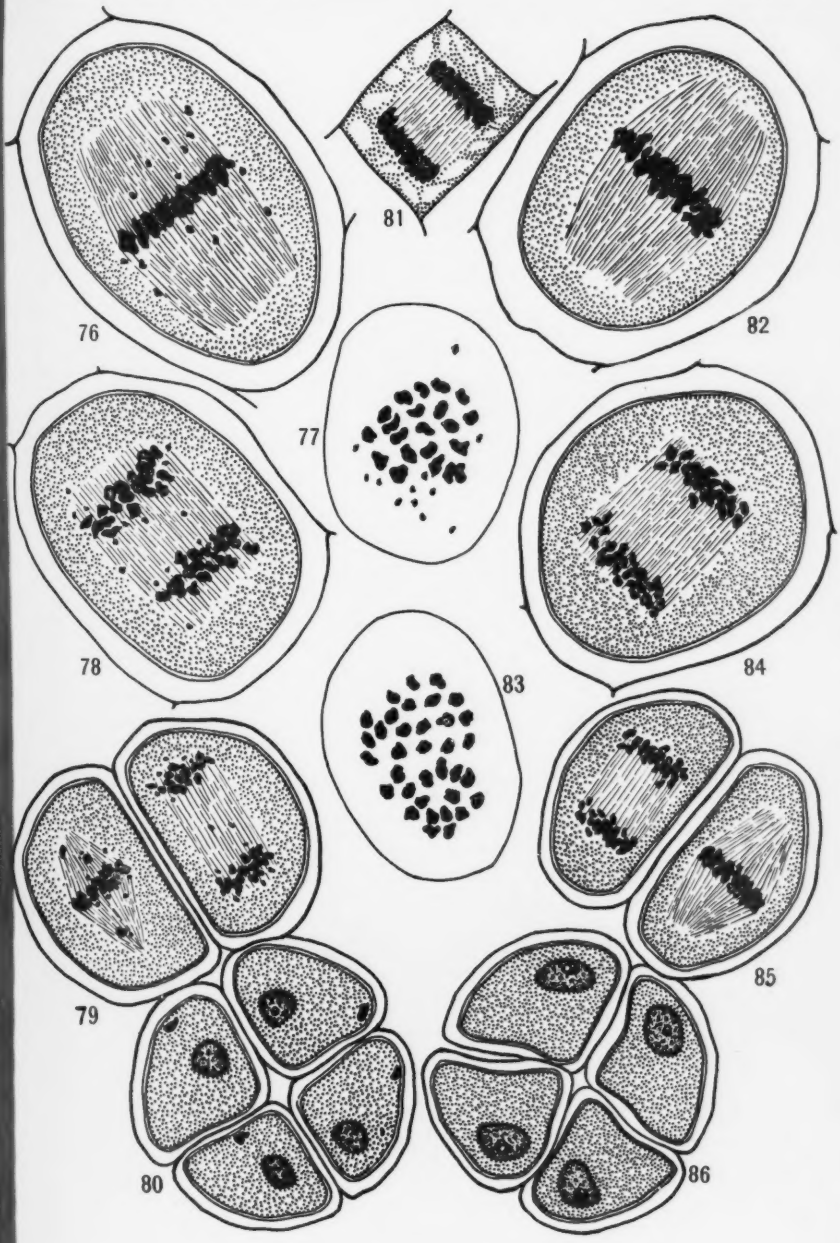

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EFFECT OF MINERAL NUTRIENTS UPON SEED PLANTS

II. PHOSPHATES[1]

THOMAS W. TURNER

Introduction

That phosphates stimulate root growth in plants is generally accepted in agricultural practice, as well as in the plant nutrition laboratory; yet the experimental evidence in support of this statement is surprisingly little. A review of the literature of the present day concerning the effects of phosphates upon plant growth shows but slight advance over the information reported from Rothamsted by LAWES and GILBERT (1) in 1847. More recent experiments reported from the same station (4, 5), and others carried on both in the field and in the laboratory at various agricultural experiment stations (2) in this country, confirm the main points of the early conclusions. SHULL (6) has brought together the main points of the phosphate problem as follows:

> In summarizing the effects of phosphates on plant growth, RUSSELL calls attention to increased growth of root system in lateral and fibrous roots and the better storage of food in root crops provided with an abundance of suitable phosphates. He mentions the influence of this element in increasing both straw and grain production, its connection with nuclear processes in cell division, its relation to normal transformations of starch, and its importance in determining the quality and feeding value of crop grown in the soil.

It might be added that investigations reported so far show that the effects of phosphates differ with the kinds of plants used, as well as with the external factors surrounding them as they grow. If we regard the general statement as demonstrated that these salts bring about an increased development of roots, we still must show whether or not this effect is due to the salt acting directly upon or

[1] Contribution from Biological Laboratory of Hampton Institute. These experiments are a part of a series begun in the Laboratory of Physiology of Cornell University, at the suggestion of Professor O. F. CURTIS, and carried on now for the past three years in the Biology Laboratory of Hampton Institute.

within the roots, or whether it may be due to some retardation in the use of the food by the aerial part which leaves it for translocation and storage or other utilization in the roots. This paper reports some results of experiments undertaken to determine whether or not the salt effects are brought about by direct effect upon the root.

Material and methods

The same general methods of water culture experimentation have been followed in this investigation as was used in earlier studies reported in detail elsewhere (**8**). An interesting feature of this method is that it conveniently makes use of nine salts, as follows:

$Ca(NO_3)_2$	KNO_3	$Mg(NO_3)_2$
$CaHPO_4$	KH_2PO_4	$Mg(PO_4)_2$
$CaSO_4$	K_2SO_4	$MgSO_4$

The use of such an arrangement of the nutrient salts makes the omission of cations or anions a simple matter, and does not necessitate introducing other so-called non-nutrient elements, which is the usual case. On the other hand, the total concentration of the solution (including the concentration of cations or anions) is lowered by this method, but preliminary studies showed that the concentrations employed had no harmful effects upon growth of the plants used. In the two solutions used (P_1, low phosphate and P_2, high phosphate), the quantity of phosphates in the high was twenty times that in the low, the other nutrients being the same in both. The salts in solution were in the following proportions:

	Low phosphate solution (per liter)	High phosphate solution (per liter)
$Ca(NO_3)_2$	0.240	0.240
$Mg(NO_3)_2 \cdot 6H_2O$	0.287	0.287
KNO_3	0.228	0.228
$CaSO_4$	0.179	0.179
$MgSO_4$	0.167	0.167
K_2SO_4	0.136	0.136
$Ca(HPO_4) \cdot 2H_2O$	0.021	0.420
$Mg_3(PO_4)_2 \cdot 4H_2O$	0.014	0.280
KH_2PO_4	0.0167	0.334
$FeSO_4$	0.033	0.033

The seeds were sterilized with formalin 1 part to 500 for 20 minutes, thoroughly washed with running tap water, and germinated between blotting paper on earthen plates. The seedlings were grown in quart jars, four to a jar.

Distilled water was used for all culture solutions, and Baker's analyzed chemicals were used throughout the experiment. The solutions were changed once a week, while the initial hydrogen concentration of the solutions as prepared ranged from pH 5.8 to 6.3. Care was taken to have the pH values the same for the low and high phosphate solutions at the beginning of each series and at each change of solution. The colorimetric method was used for determining the pH values, and the adjustments of the same were made with N/20 NaOH or 0.1 per cent HCl when it was necessary to bring the solutions to the same pH. The osmotic pressures of the two solutions used were not determined, but there was no indication of any injury in growth due to the greater concentration of the high phosphate solution.

The method of getting quantitative data was the same as that reported in a former paper (**8**). In brief, while other salt quantities than phosphates were kept approximately constant, the ratios of growth of tops to growth of roots for particular periods were determined by weight. Any marked variation in the ratio of tops to roots under the conditions would be attributed to the varying quantities of phosphates in the two solutions. The second question to be determined, namely, whether or not this variation was the result of an accelerating effect of the salt acting directly upon the roots, was approached through the employment of Robbin's method of growing root tips under sterile conditions in the solutions already described. Dextrose to the amount of 1 per cent was added to the solutions to supply the necessary carbohydrates. Barley, wheat, and cotton were the plants used, but the root tips of corn only were tried.

Results

Experiment 1: barley

This experiment extended over 23 days and was divided into two series, one covering a 16-day period, the other extending over

the entire 23 days. In all cases the cultures were set up at the same time and were grown under the same greenhouse conditions. Four jars, making a total of sixteen plants, were harvested and weighed at each series. Table I gives the results of the first set of experiments dealing with barley seedlings.

TABLE I

EFFECT OF INCREASING PHOSPHATE CONCENTRATION ON RATIO OF TOP TO ROOT GROWTH IN BARLEY SEEDLINGS (GREEN AND DRY WEIGHTS IN GRAMS)

		Series I (16 days)			Series II (23 days)		
		Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots
Solution 1, low phosphate...	Green weight	4.3204	0.9313	4.64	5.5533	1.0668	5.21
		4.318	0.8358	5.17	4.9135	0.9405	5.22
		3.4212	0.7732	4.42	5.7146	1.0713	5.33
		3.5964	0.7860	4.58	4.9290	0.9100	5.42
	Total	15.6560	3.3263		21.1104	3.9886	
	Mean	.....	.....	4.70			5.30
	Dry weight	0.3446	0.0495	6.96	0.4641	0.0605	7.67
		0.3318	0.0528	6.28	0.4435	0.0630	7.04
		0.2790	0.0463	6.03	0.5000	0.0670	7.46
		0.2664	0.0482	5.53	0.4368	0.0558	7.83
	Total	1.2218	0.1968		1.8444	0.2623	
	Mean	.....	.....	6.20			7.50
Solution 2, high phosphate...	Green weight	3.5263	0.9070	3.89	4.2060	0.9824	4.28
		3.0346	0.6814	4.45	4.2537	0.8671	4.91
		2.6900	0.7339	3.67	5.8868	1.0410	5.65
		3.6600	0.7158	5.11	3.6552	0.9544	3.83
	Total	12.9109	3.0381		18.0017	3.8449	
	Mean	.....	.....	4.28			4.67
	Dry weight	0.2906	0.0583	4.98	0.3797	0.0758	5.01
		0.2440	0.0426	5.73	0.3707	0.0655	5.66
		0.2324	0.0518	4.49	0.4839	0.0763	6.34
		0.2922	0.0543	5.38	0.3200	0.0690	4.64
	Total	1.0592	0.2070		1.5543	0.2866	
	Mean	.....	.....	5.15			5.41

In series I (16-day period), the mean of the ratios of top to root growth for the four cultures was 6.20 for the dry weight in the low phosphate solution, and 5.15 for those in the high phosphate. The difference between them is 1.05. The green weights showed ratios in the same order, although not quite so marked, being 4.70 for low and 4.28 for the high phosphate. In series II (23-day period) the mean ratios for the dry weights were 7.50 for the low phosphate and 5.41 for the high phosphate, the difference being 2.09.

GROWTH CHARACTERS.—The high phosphate cultures were more rigid and the leaves more erect, otherwise they appeared equally green and healthy. No experiments have been attempted so far to show whether or not the differences in rigidity and erectness of these plants may be explained on an anatomical basis or on the basis of a difference in concentration of the solution surrounding the cells. It will be seen as one compares the difference in these ratios that

TABLE II

EFFECT OF INCREASING PHOSPHATE CONCENTRATION ON RATIO OF TOP TO ROOT GROWTH IN WHEAT SEEDLINGS (GREEN AND DRY WEIGHT IN GRAMS)

		Series I (15 days)			Series II (22 days)			Series III (29 days)		
		Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots
Solution 1, low phosphate	Green weight	2.6586	1.1420	2.33	5.7944	1.3634	4.25	5.7435	1.1400	5.04
		3.1630	1.3442	2.35	4.4580	1.2834	3.47	5.5158	1.1738	4.7
		3.2848	1.4286	2.3	4.4528	1.3102	3.39	6.5370	1.9618	6.1
		2.744	1.2746	2.15	5.7403	1.5470	3.71	5.8019	1.5153	4.3
	Total	11.8308	5.1794		20.4455	5.5040		23.5982	4.7909	
	Mean	.....	.....	2.3			3.7			5.03
	Dry weight	0.2971	0.0714	4.15	0.5634	0.0955	5.90	0.6444	0.0918	7.0
		0.3317	0.0784	4.23	0.4611	0.0820	5.62	0.6714	0.0975	6.8
		0.3566	0.0884	4.03	0.4889	0.0980	4.99	0.6778	0.0916	7.4
		0.3030	0.0727	4.16	0.5627	0.1043	5.39	0.6454	0.1207	5.3
	Total	1.2884	0.3109		2.0761	0.3798		2.6390	0.3916	
	Mean	.....	.....	4.1			5.4			6.6
Solution 2, high phosphate	Green weight	2.7163	1.4828	1.83	5.9147	1.5232	3.88	6.3349	1.4203	4.48
		2.8432	1.4268	1.99	5.6276	1.2438	4.52	6.2000	1.1125	5.57
		3.0169	1.5755	1.90	4.4532	1.4182	3.14	7.4203	1.7893	4.14
		2.6996	1.7705	1.50	6.1466	1.4132	4.35	7.5750	1.7514	4.32
	Total	11.2760	6.2556		22.1421	5.5984		27.5302	6.0735	
	Mean	.....	.....	1.8			3.9			4.9
	Dry weight	0.2847	0.0920	3.09	0.5610	0.1382	4.06	0.7025	0.1282	5.55
		0.3000	0.0861	3.48	0.5489	0.1215	4.51	0.6587	0.1429	4.60
		0.3220	0.0939	3.42	0.4356	0.1114	3.91	0.7751	0.1574	4.92
		0.2094	0.1054	2.84	0.5776	0.1168	4.94	0.7846	0.1637	4.79
	Total	1.2061	.3274		2.1221	0.4879		2.9409	0.5922	
	Mean	.....	.....	3.2			4.3			4.96

there is a 100 per cent increase in case of the 23-day period over the 16-day for the dry weights. The higher phosphate concentration makes for decreasing ratio of tops to root. Nitrates were demonstrated to have the opposite effect in barley (8).

EXPERIMENT II: WHEAT

The duration of this experiment was 29 days. The pH value in case of these culture solutions when prepared was brought in each case to 5.8.

GROWTH CHARACTERS.—No apparent differences were noted before or after harvesting in size and color of plants in the cultures;

all showed a healthy green. The high phosphate plants stood more erect and were more rigid, as was noted in case of barley. The results of the three series of this experiment are given in table II, where both dry and green weights show (with one exception) that ratios decrease with the increase of phosphates in the solution. For the 22-day period it will be noticed that there is an increase of 0.2, rather than a decrease, in the ratio for the green weights; but other than this, all remaining figures point in the same direction. The top root ratios based upon dry weights for the three series of this experiment are as follows:

	Solution 1	Solution 2
Series I (15 days)	4.1	3.2
Series II (22 days)	5.4	4.3
Series III (29 days)	6.6	4.96

Experiment III: cotton

The solution cultures with cotton were set up in the same way as those of barley and wheat, except that only three plants were set in a quart jar and three jars instead of four were harvested to a series. The duration of this experiment was 48 days. The solutions were changed weekly, and the pH values of the new solutions were found to vary from 5.8 to 6.2; but, as just stated, the two solutions were always brought to the same pH for each renewal.

The line of demarcation between root and stem is not clear in cotton, but by allowing a longer time before beginning to harvest one can determine this with greater accuracy. Two series were conducted with this plant, the first harvested after 40, the second after 48 days. The dry weights only are recorded. The differences in the ratios of tops to roots of plants growing in solution 1 and solution 2 are not striking, but the results are similar to those of experiment I and experiment II, as table III will show.

While the plants growing were healthy looking and green, and apparently the same size, the dry weights showed decidedly greater growth for the low phosphate solutions. There is a possibility that the high phosphate solution used is too near the maximum concentration for the optimum growth for young cotton seedlings. This question is being tested in our laboratory.

Experiment IV

The three foregoing experiments with barley, wheat, and cotton show unmistakably that the ratio of top to root growth in these plants is affected by the concentration of phosphates in the solution, and that the effect was the opposite of that which has been shown to result from nitrate treatment in increasing quantities. In every case the dry weights showed a decrease in the ratios as phosphates were increased. This was true of green weights also, with a single doubtful exception.

TABLE III

Effect of increasing phosphate concentration on ratio of top to root growth in cotton seedlings (dry weight in grams)

		Series I (40 days)			Series II (48 days)		
		Tops	Roots	Tops / Roots	Tops	Roots	Tops / Roots
Solution 1, low phosphate...	Dry weight	2.2326	0.2164	10.32	2.4462	0.2053	11.92
		1.7854	0.1781	10.03	2.3346	0.2074	11.26
		1.9440	0.2176	8.93	2.8945	0.2473	11.30
	Total.....	5.9620	0.6121		7.6753	0.6600	
	Mean.....	.....	.....	9.76			11.49
Solution 2, high phosphate...	Dry weight	1.5754	0.1639	9.61	1.8073	0.2076	8.71
		1.9432	0.2081	9.34	2.0010	0.2397	8.35
		1.7916	0.2354	7.61	1.9287	0.2308	8.34
	Total.....	5.1112	0.6074		5.7370	0.6781	
	Mean.....	.....	.....	8.85			11.13

Since the results found in these experiments indicate greater relative increase of roots, experiment IV was conducted with the aim of determining whether or not this increase is due to the effect of the salt acting directly upon the roots.

Robbin's (3) method of growing root tips in sterile cultures was followed. The seeds (corn only being used) were carefully selected, sterilized in $HgCl_2$ (1–1000) for 45 seconds, washed thoroughly in sterile distilled water, and germinated on sterile agar in 250 cc. wide-mouthed extraction flasks. When the roots of the germinating seeds reached a length of 3 cm. or more they were removed under sterile conditions and transferred to the prepared sterile culture solutions. The pH values of lots of the solutions were tested before and after sterilization and no change was observed. The lengths were

carefully measured, after which they were set away in a dark chamber in the greenhouse to grow. Increase in length and number of secondary roots produced were the chief points considered. Other

TABLE IV

EFFECT OF INCREASING PHOSPHATE ON GROWTH OF ROOT TIPS OF CORN UNDER PURE CULTURE CONDITIONS

	Solution 1, low phosphate				Solution 2, high phosphate		
Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters	Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters
	Series I (19 days)						
1	3.0	8.5	19 LD*	1	4.0	12.5	25 SD
2	6.0	11.5	106 SD	2	2.0	8.0	18 SD
3	4.5	12.4	45 LD	3	3.0	5.3	5 SD
4	5.0	9.3	68 SD	4	4.0	7.0	42 SD
5	7.0	12.3	100 LD	5	4.5	6.2	14 SD
6	5.5	13.0	71 SD	6	6.0	10.4	16 LD
7	4.0	12.6	75 SD	7	5.0	7.3	51 SD
8	6.0	16.6	134 LD	8	5.0	6.5	12 SD
9	6.0	10.5	76 SD	9	3.5	7.2	18 SD
10	5.0	16.3	85 LD	10	6.0	8.4	65 SD
11	5.5	12.0	73 SD	11	3.0	5.3	7 SD
12	3.5	13.5	60 SD	12	3.0	5.8	9 SD
13	4.5	8.3	17 SD	13	8.0	11.6	56 SD
14	4.5	17.0	54 LD	14	7.0	9.0	62 SD
15	6.5	13.7	124 SD	15	5.5	8.8	60 SD
16	4.0	10.0	51 LD	16	4.5	8.0	27 SD
17	5.0	10.5	55 SD	17	5.5	8.0	63 SD
Total	85.5	208.0	1213		83.5	135.3	550
Average per culture	5.03	12.2	71.35		4.9	7.88	32.3
Percentage increase in length	.....	137				60.8	
	Series II (16 days)						
1	3.5	16.3	45 LD	1	5.0	7.3	34 SD
2	6.3	15.3	71 LD	2	3.5	6.5	33 SD
3	3.3	17.8	37 LC	3	4.5	7.4	14 SD
4	7.0	15.8	97 LD	4	4.0	7.2	19 SD
5	3.6	14.2	96 SD	5	5.3	7.8	45 SD
6	4.7	14.8	82 SD	.....	.....	.....	.....
7	6.5	14.2	74 SC	.....	.....	.....	.....
8	3.3	16.8	15 LC	.....	.....	.....	.....
9	6.5	20.3	89 LD	.....	.....	.....	.....
Total	44.7	145.5	606		22.3	36.2	165
Average per culture	4.96	16.6	67.3		4.46	7.24	29
Percentage increase in length	.....	234.6				62.3	

TABLE IV—*Continued*

Solution 1, low phosphate				Solution 2, high phosphate			
Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters	Culture no.	Initial length in cm.	Final length in cm.	No. of secondary roots and growth characters
	Series III (16 days)						
1	5.2	11.5	58 SD	1	3.5	5.4	20 SD
2	3.8	7.0	21 SD	2	6.0	7.2	67 SD
3	5.2	13.1	69 SD	3	7.8	9.6	49 SD
4	6.0	15.8	83 LD	4	3.5	5.0	25 SD
5	9.2	16.1	115 SD	5	3.3	5.1	20 SD
6	4.5	12.4	99 SD	6	6.8	9.0	63 SD
7	5.2	14.7	58 LD	7	6.2	7.3	51 SD
8	7.6	16.3	48 LD	8	7.6	11.0	91 SD
9	3.8	15.0	61 SD	9	4.6	7.3	21 SD
10	6.0	13.4	61 SD	10	8.3	14.0	101 SD
11	6.6	11.0	65 SD	11	4.8	6.5	23 SD
12	3.4	13.4	44 LD	12	4.0	5.2	22 SD
Total	66.5	159.7	782		66.4	92.6	553
Average per culture	5.5	13.3	65.1		5.5	7.7	46.0
Percentage increase in length	.....	141.8				40	

* L (long) denotes secondary roots greater than 0.75 cm.; D denotes uniform distribution of secondary roots along growing tip; S (short) denotes secondary roots, equal to or less than 0.75 cm.; C (crowded) denotes tufting of secondary roots around cut end.

growth characters were noted, including the manner of growth of the secondary roots, whether long or short, and crowded toward the cut ends or distributed throughout the length of the root.

Three series of these pure culture experiments were conducted, running for 19, 16, and 16 days respectively, as shown in tables IV. It will be seen that the number of cultures which figure in the results is not the same for the different series. In series II the number is smaller, as several cultures had to be discarded because of contamination. In series I (17 cultures) the average length of root tips in the low phosphatic solution at the beginning was 5.03 cm.; at end of 19 days it was 12.2 cm. The increase in length during this period was 133 per cent. The average number of secondary roots was 71.35.

For the seventeen cultures in the high phosphate solution the average initial length was 4.9 cm., which increased to 7.88 cm. in 19 days, or 57.9 per cent. Also, for this first series the average num-

ber of secondary roots, which was found to be 71.35 per culture for the low phosphate solution, was only 32.3 for the high. In series II we have not the same number of cultures in the solutions for comparison (nine cultures in solution 1 and five in solution 2), but, with this reservation, we find the average of results similar to those for series I. In series III we have a still closer similarity of results to those of series I. As one studies the three series in table IV, it becomes clear that under the conditions of this experiment, increasing the concentration of phosphates in the nutrient solution neither brings decreased growth in length nor increase in the number of secondary roots. The growth features of the sterile cultures showed no marked differences. Strikingly long secondary roots occurred seldom in high phosphate cultures, but were of frequent occurrence in the low phosphate.

Discussion

It is well known that phosphates play varying rôles in the living plant. Their beneficial effects upon underground portions of certain plants growing under field conditions have been observed for nearly a century, but there is little to be found in the literature to indicate that these field results have been followed up by laboratory experiments with sufficient definiteness to determine the exact way in which phosphates function in the plant. The water culture experiments reported here for barley, wheat, and cotton (tables I–III) show without question that the ratios of top to root growth decrease in each case as the phosphate concentration of the solution increases. One may interpret such a result to mean either that the salt has a retarding effect upon the tops or a stimulating effect upon the roots. LAWES and GILBERT stated many years ago: "It is certainly true that it (super-phosphate of lime) causes a much inhanced development of the underground collective apparatus of the plant especially of lateral and fibrous roots." Sir JOHN RUSSEL (4) in 1921 wrote: "In the absence of phosphorus, swedes and turnip roots will not swell, but will remain permanently dwarfed." These quotations, the old and the new, point to some direct effect of the salt upon the underground parts as the explanation.

The pure culture experiments reported in the three series of table IV are strikingly constant in showing that increasing the phosphate concentration not only does not have the effect of stimu-

lating directly growth in length or multiplication of lateral roots, but that both of these are retarded under such conditions. Corn root tips only have been dealt with so far in these experiments. A summary of table IV shows the results more clearly.

The experiment shows for example that cellular activity which should manifest itself in increased growth in length or multiplica-

TABLE IV—SUMMARIZED

	Solution 1		Solution 2	
	Percentage increase in length	Average no. secondary roots per culture	Percentage increase in length	Average no. secondary roots per culture
Series I, 19 days	133	71.35	57.9	32.3
Series II, 16 days	234	67.3	62.3	29
Series III, 16 days	141.9	65.1	40	46

tion of secondary roots is not increased by direct application of phosphates, as is implied in the usual statements. The actual fact noted, therefore, that there is a decreasing ratio of tops to roots as the phosphate concentration is increased, must find explanation in the formation of compounds or simple substances in connection with photosynthetic activity in the tops, which are translocated to the roots and manifest themselves there by their growth stimulating or storage effects.

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EFFECT OF NITRATE SALTS UPON GROWTH AND COMPOSITION OF TOBACCO LEAVES[1]

A. R. C. HAAS

(WITH ONE FIGURE)

Nitrate fertilization of tobacco has been shown by VALLEAU and JOHNSON (10) to be efficacious in bringing about the recovery of tobacco leaves from frenching. The writer has been able to produce chlorotic-appearing tobacco leaves in experiments by using 10-gallon containers filled with Sierra loam which was known to contain a very small concentration of nitrate. VALLEAU and JOHNSON were able to bring frenched plants back to normal growth in soil to which was added a complete nutrient or nitrogen as sodium, potassium, calcium nitrate, or ammonium sulphate, etc.

It seemed of interest to consider solely the effect of the cation added to the soil when the nitrate salt was used in each case. Ammonium, sodium, potassium, calcium, and magnesium nitrate were used respectively for the various soil cultures. Each day the soils were given 3 liters of their respective nitrate solutions, made up with distilled water, the nitrate concentration in each case being about 2000 p.p.m. After several weeks of such applications, during which time and subsequent thereto the replaced bases were free to escape in the drainage water, the concentrations of the solutions applied were reduced gradually. At the time (July 8) of transplanting the young Kentucky broad-leaf tobacco plants from the seed bed to the experimental containers, the solutions applied contained about 500 p.p.m. of nitrate. In this manner the soil was changed considerably in its base exchange complexes, and yet with but one exception moderate growth was still permitted. In the sodium nitrate-treated soil the plants were able to maintain slight growth if they were raised on little mounds of soil at the time of being transplanted. By doing this none of the black surface solution, which drained away

[1] Paper no. 195, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

slowly, came into direct contact with the tender portions of the plant. Notwithstanding these precautions the plants in the sodium nitrate-treated soil grew so slowly that they were excluded from the experiment. Tobacco plants were transplanted into some untreated Sierra loam soil as a control and received only distilled water. One such control was given the sodium nitrate treatment in order to give the plants opportunity to become well established prior to the occurrence of severe alterations in the nature of the base exchange complex. In spite of the large and repeated applications of solution, the growth was excellent. There was also a moderate growth in the other treated soils.

The most important effect observed was in the leaves of the plants in the ammonium nitrate-treated soil. Yellowish areas occurred between the veins, as shown in fig. 1. This was first noticeable in the lowest leaves, and progressively made its appearance in leaves higher up as they became mature. The yellow areas were generally distributed throughout the entire length of the blade, and gave the leaves a mottled appearance. This differs from the chlorosis that GARNER, MCMURTREY, and MOSS (2) have shown to result from a magnesium deficiency in which the chlorosis begins at the tip and along the outer margins of the oldest tobacco leaves and advances toward the leaf base (cf. JOHNSON 4). The effect produced upon the leaves by the ammonium nitrate treatment of the soil also differs from that produced by a deficiency of potassium, as described by MOSS, MCMURTREY, LUNN, and CARR (6), in which the leaves are puckered and rough, with mottling beginning at the tips, often followed by the appearance of small centers of dead tissue.

Further differences between the effects of ammonium and that of a deficiency of magnesium or potassium are obtained by analyzing the leaves of the tobacco plants taken from the variously treated soils. The most mature leaves of the plants in the various cultures were collected on November 23, dried, and ashed. Table I shows the analyses of the ash of the various leaf samples.

It will be seen that for the plants grown in the soil treated before the planting was made, the ash, as a percentage of dry matter, is greatest in the control plants. The sodium nitrate treatment of the soil was not begun until after the planting was made, and the per-

centage of ash in this case was slightly greater than that of the control plants. After the base exchange complex of the soil has been

FIG. 1.—Tobacco leaf from plant grown in ammonium nitrate-leached soil

acted upon by a single salt solution until replacement and leaching away of the replaced bases has progressed to a considerable degree, the ash of the leaves, as a percentage of the dry matter, becomes lower than that of the control. When saturation of the base exchange complex of the soil with a single base is approached, deficiencies of various bases no doubt may enter as factors in the growth of the plants.

The calcium, as a percentage of the dry matter, was greatest in the leaves of the plants from the sodium nitrate-treated soil, followed

TABLE I

ASH COMPOSITION OF TOBACCO LEAVES IN RELATION TO SOIL TREATMENT

SOIL TREATMENT	ASH AS A PERCENTAGE OF DRY MATTER	ASH CONSTITUENTS							
		Percentage of dry matter (calculated from ash analyses)				Percentage of ash			
		Ca	Mg	Na	K	Ca	Mg	Na	K
Sodium nitrate.....	18.85	4.45	1.50	1.41	1.59	23.59	7.98	7.47	8.42
Calcium nitrate.....	11.02	2.87	0.29	0.34	1.58	26.07	2.65	3.07	14.34
Magnesium nitrate..	7.99	0.78	1.93	0.47	1.01	9.79	24.10	5.85	12.64
Ammonium nitrate..	5.55	0.78	0.30	0.19	1.43	14.11	5.46	3.47	25.78
Ammonium nitrate..	7.85	0.99	0.32	0.65	1.83	12.60	4.05	8.27	23.32
Control (distilled water).....	18.39	3.34	0.55	0.59	3.62	18.16	3.00	3.20	19.68
Calcium nitrate.....	15.46	3.50	0.32	0.67	2.87	22.66	2.07	4.34	18.55
Potassium nitrate...	16.68	0.86	0.28	1.63	7.58	5.16	1.69	9.79	45.47

in order by the calcium nitrate-treated, and the control; whereas the ammonium, potassium, and magnesium-treated showed a marked reduction below that of the control. The low value in the one case of calcium nitrate is due to the yellowing and drying up of the mature lowest leaves, and hence is not strictly comparable with the other cultures in this regard. The marked reduction in the percentage of calcium in the dry matter of the leaves of the plants from the ammonium, potassium, and magnesium-treated soil as compared with the control is very striking. This is no doubt a result of the replacement of the calcium from the base exchange complex of the soil by the base of the added salt solution, and the subsequent leaching out of the replaced calcium and other bases in the drainage water.

The values of the magnesium, as a percentage of the dry matter, are not very great even for the plants in the magnesium-treated soil. There was a reduction below that of the control in the plants in the calcium, ammonium, and potassium-treated soil, but an increase over that of the control in the plants in the sodium nitrate-treated soil. Notwithstanding the large additions of sodium nitrate to the soil, the leaves contained relatively little sodium, since as large a percentage of sodium was found in the dry matter of the leaves of the plants in the potassium-treated as in the sodium-treated soil. All treatments decreased the percentage of potassium in the dry matter as compared with the control, with the exception of the plants in the potassium-treated soil where a large increase was noted.

The calcium, as a percentage of the ash of the leaves, is reduced by the potassium, magnesium, or ammonium treatment. The dry matter of the leaves of the plants in the magnesium-treated soil contained a relatively low percentage of magnesium, but because of the small percentage of ash in the dry matter, the percentage of magnesium constitutes about one-fourth of the ash. The ash of the plants in the potassium-treated soil contained over 45 per cent of potassium. Of the cultures receiving no potassium, the ash of the leaves from the sodium nitrate-treated soil contained the least, and those from the ammonium-treated the largest percentage of potassium.

Having considered the effects that the added bases in the nitrate solution had upon the composition of the leaves, the effects observed in the leaves of the plants in the ammonium nitrate-treated soil can hardly be due either to a deficiency of magnesium or potassium, but appear to be an effect of the ammonium base of the added solution. For if we consider the magnesium as a percentage of dry matter, we find that the results for the leaves of the plants in the calcium- and in the potassium-treated soil are as low as those of the ammonium-treated, and yet have none of the leaf symptoms as found in the ammonium-treated. The magnesium as a percentage of ash is higher in the leaves of the plants from the ammonium-treated soil than in all but the sodium and magnesium-treated. Consequently, the effect produced on the plants in the ammonium-treated soil cannot be attributed to magnesium deficiency or "sand drown" (1).

If we consider the percentage of potassium in the dry matter,

the values found for the leaves of the plants in the ammonium-treated soil are not unlike those found for the leaves of the plants in the sodium, calcium, or magnesium-treated soil where no effects were observed. The potassium, as a percentage of ash, is greater in the leaves of the plants from the ammonium-treated soil than in the leaves of the plants on any other treated soil except the potassium-treated, and, therefore, a deficiency of potassium cannot be regarded as the cause of the symptoms observed in the leaves of the plants in the ammonium-treated soil (6). It is concluded, therefore, that when the base exchange complex of the soil is largely saturated with the ammonium base, the soil solution in equilibrium with the nearly saturated ammonium soil complex becomes toxic to the tobacco leaves and produces the effects observed. The toxic effect of ammonium ions for certain plants has been shown by SÖDERBAUM (7, 8, 9).

It is of interest to note that none of the leaves of the plants in the nitrate-treated soil showed frenching. This known relationship, and the observations of MORRIS (5) that legumes when plowed under have brought about the recovery of rosetted pecan trees, have led VALLEAU and JOHNSON (10) to suggest that the frenching of tobacco leaves and the rosette of pecan trees have a common cause, namely, a deficiency of available nitrogen. There is, however, no experimental evidence to show that the plowed-under legumes brought about the recovery as a result of the nitrogen in the legumes. MORRIS in fact showed that applications of sodium nitrate, dried blood, or manure gave no improvement. HAAS, BATCHELOR, and THOMAS (3) have reported severe cases of rosette of pecan trees in experimental plots at the Rubidoux tract of the Citrus Experiment Station, where the sources of nitrogen were sodium nitrate, dried blood, and steamed bone meal.

When previously untreated Sierra loam soil was placed in two wide sewer pipes placed one above the other, and planted to pecan trees and leached every day with potassium and magnesium nitrate solution respectively, severe cases of pecan rosette developed. In these unpublished results on pecan trees and on the mottling of citrus, the writer finds these physiological diseases to be independent of the nitrogen portion of the added salt solution.

Summary

1. Frenching of tobacco leaves occurs when nitrate is deficient but disappears upon the application of sufficient nitrogen to the soil.

2. The continual application of nitrate solutions to soil prior to and during the growth of tobacco plants affects the base exchange complex in the soil, and consequently the bases absorbed by the leaves. Such continual applications of ammonium nitrate bring about toxic conditions in the tobacco leaves.

3. The recovery of tobacco from frenching by the application of nitrate does not appear to bear any direct relationship to the recovery of pecan trees from rosette following the use of legumes.

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STUDIES IN CALIFORNIAN HEPATICAE

II. FOSSOMBRONIA LONGISETA

ARTHUR W. HAUPT

(WITH PLATE VII)

In a former investigation of *Fossombronia cristula*, a species ranging from New England to Indiana, the writer (3) found several features in disagreement with HUMPHREY'S (4)[1] earlier study of *F. longiseta*, a form widely distributed in California. The chief point of variance in the two papers concerns the antheridium, HUMPHREY describing a unique development allying *Fossombronia* with *Sphaerocarpus* and *Geothallus* rather than with the other Jungermanniales. The writer, on the other hand, found the development of the antheridium in no way peculiar, but like that characteristic of other members of the order, as LEITGEB (5) had previously reported for *Fossombronia pusilla*.

With respect to the development of the antheridium, the Marchantiales and Jungermanniales stand in striking contrast to each other, and yet within each order this development is remarkably uniform, as is well known. Consequently to find within a single genus a wide variation in regard to this fundamental phase of the life history would indeed be an extraordinary circumstance. The present study of *Fossombronia longiseta* was undertaken particularly to attempt to determine whether the development of the antheridium in this species is really unique, as reported by CAMPBELL and by HUMPHREY, or whether it is like that of *Fossombronia cristula* previously described by the writer. Incidentally certain noteworthy details concerned with the development of the archegonium and of the

[1] In the 1905 edition of CAMPBELL'S *Mosses and ferns* is found a brief statement regarding the development of the antheridium of *Fossombronia longiseta*, a short description of the early stages in embryogeny, and three sets of figures drawn for the book by HUMPHREY showing the development of the antheridium, archegonium, and embryo. HUMPHREY'S own paper (4) did not appear until the following year, and so to CAMPBELL should belong credit for the first published account of the development of this species.

embryo were discovered, and these are included in the present paper. No attention is given to such other phases of structure and development as are adequately described in the literature cited.

Material

Most of the material used in this investigation was obtained during 1925–1928 from several different localities in the San Gabriel Mountains of Los Angeles County, chiefly from Santa Anita Canyon. One collection was also made in 1928 at the foot of Bridal Veil Falls in Yosemite Valley. So far as the features dealt with in this study are concerned, no differences could be observed between the plants collected in Yosemite Valley and those from southern California. The only figure drawn from the Yosemite material is fig. 2.

The most favorable material to illustrate the development of the sex organs was collected in November and December. Like other liverworts, the plants dry up during the long rainless summer. Collections were also made throughout the winter and early spring, but young stages in the development of the sex organs were more difficult to find at this time than in the late autumn.

Sex organs

Fossombronia longiseta is monoecious, the antheridia and archegonia being more or less separately grouped. Frequently they are somewhat intermixed, however, a feature which makes it very difficult to distinguish between the two kinds of sex organs when young. In general there are relatively more antheridia produced when the plants are young and relatively more archegonia later. The sex organs develop acropetally from the dorsal segments of the dolabrate apical cell, but occasionally they may arise farther back among the older ones.

Antheridium

The antheridium develops from a papillate initial which undergoes a transverse division into a large basal cell and a smaller outer cell. As in *Fossombronia cristula*, another transverse wall appears, probably in the outer cell, in agreement with other members of the order. In *Pallavicinia* the writer (2) has demonstrated this exact

sequence of wall formation in the young antheridium. Of the three cells present, the upper one is the primary antheridial cell, the middle one forms the stalk, and the lower one remains imbedded in the thallus (fig. 1).

The primary antheridial cell soon undergoes a median vertical division, and the primary stalk cell usually divides at the same time, either vertically (figs. 1, 2) or transversely (fig. 4). The first vertical division of the primary antheridial cell is not followed by another vertical one at right angles to the first, as described by HUMPHREY (4); but four periclinal walls are formed in the primary antheridial cell, arranged as shown in fig. 3. The young antheridium now consists of an imbedded portion, a stalk, and two spermatogenous cells surrounded by four primary wall cells, exactly as in *Fossombronia cristula* and other anacrogynous Jungermanniales. In no case were periclinal walls seen in the second tier of cells, another feature described by HUMPHREY. The two primary spermatogenous cells stain more deeply than the cells of the wall and stalk, and thus are easily recognized. Further growth of the antheridium is typical in every way, and need not be described.

HUMPHREY's account of the development of the antheridium of *Fossombronia longiseta* differs from that described here in two main respects: (1) The primary antheridial cell and the cell just below it divide by vertical walls arranged at right angles to each other, resulting in the formation of an octant. (2) Periclinal wall formation, differentiating the primary spermatogenous cells from the sterile jacket, involves the entire superficial portion of the young antheridium, that is, two tiers of cells. A complete discussion of HUMPHREY's account is given in the writer's paper on *Fossombronia cristula* (3), and nothing more need be said here than that the present study has yielded no evidence whatsoever in support of his interpretation of the development of the antheridium.

ARCHEGONIUM

Like the antheridium, the archegonium arises from a papillate initial usually formed very close to the apical cell. A transverse division results in the formation of a large basal cell and a smaller outer cell (fig. 5), although HUMPHREY finds that the outer cell is

considerably the larger. HUMPHREY also states that the archegonium initial does not extend so far above the surrounding cells as does the antheridium initial, but no evidence of this was seen by the writer. The basal cell may divide again transversely, or it may remain undivided until three vertical walls have appeared in the outer cell in the way characteristic of all Hepaticae (figs. 5, 6). HUMPHREY reports that the basal cell does not undergo another division until after the vertical walls have appeared in the outer cell. This is certainly not invariably the case. The behavior of the basal cell, as here reported, corresponds exactly with that of *Fossombronia cristula* previously described by the writer (3).

The division of the primary axial cell to form a cover cell and a central cell (fig. 7), and the formation by the latter of a primary neck canal cell and a ventral cell (fig. 8) are in every way typical. The primary neck canal cell soon gives rise to an upper and a lower cell (fig. 8). Both of these may divide again (fig. 11), the upper one dividing first (figs. 9, 10). More frequently, however, the lower neck canal cell may fail to divide. The cover cell divides by a vertical wall most commonly soon after the division of the primary neck canal cell has taken place. The ventral cell now forms the ventral canal cell and the egg (fig. 12), while the two upper neck canal cells may give rise to four (fig. 13).

Of the two cells derived from the primary neck canal cell, the lower one may remain undivided while the upper one divides once before the division of the ventral cell. This results in an archegonium with three neck canal cells (fig. 12). In other cases the lower cell resulting from the division of the primary neck canal cell may remain undivided while the upper one divides twice, thus resulting in an archegonium with five neck canal cells (fig. 13). Sometimes the lower cell divides once and the upper one twice, thus producing six neck canal cells (fig. 15). Various intermediate conditions may occur (fig. 14).

In *Fossombronia cristula* the writer found that 6–8 neck canal cells are formed. In *F. longiseta*, HUMPHREY states that "the number of neck canal cells is, so far as observed, invariably six." Since his fig. 44 represents an archegonium with an egg, ventral canal cell, and two neck canal cells, and his fig. 45 shows one with six neck

canal cells, it is evident that the number may be increased after the ventral canal cell and egg are differentiated. HUMPHREY does not, however, refer to this fact.

Embryo

While no attempt was made to work out a complete series of stages in the development of the embryo, enough stages were seen to indicate that the embryogeny of *Fossombronia longiseta* is essentially the same as described by CAMPBELL (1) and HUMPHREY (4). Most of the embryos observed consisted of four tiers of cells, each tier being composed of two or four cells. In *F. cristula* the writer (3) found 6–8 superimposed cells before the appearance of the vertical walls. Thus in *F. longiseta* the vertical divisions occur much earlier.

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EXPLANATION OF PLATE VII

The magnification in each case is 500 diameters.

FIG. 1.—Two young antheridia, the one to left consisting of basal cell, stalk cell, and primary antheridial cell; the one to right shows periclinal wall formation in antheridial cell and division of basal and of stalk cells by vertical walls.

FIG. 2.—Slightly older antheridia; in one to right the primary wall cells have divided, in other one the primary spermatogenous cells have increased to four; in both cases the basal cell has remained undivided.

FIG. 3.—Cross-section of young antheridium showing two primary spermatogenous cells surrounded by four primary wall cells.

FIG. 4.—Young antheridium showing formation of stalk.

FIG. 5.—Two young archegonia; the one to left shows division of initial to form large basal cell and smaller outer cell; in one to right a horizontal wall has appeared in basal cell and vertical wall in outer cell.

FIG. 6.—Formation of vertical wall in stalk and of second vertical wall in outer cell.

FIG. 7.—Formation of cover cell and central cell from primary axial cell, central cell about to divide.

FIG. 8.—Formation of primary neck canal cell and ventral cell from central cell, primary neck canal cell undergoing first division.

FIG. 9.—Division of cover cell by vertical wall and formation of three neck canal cells, lower one not yet divided.

Fig. 10.—Later stage showing division of lower neck canal cell.

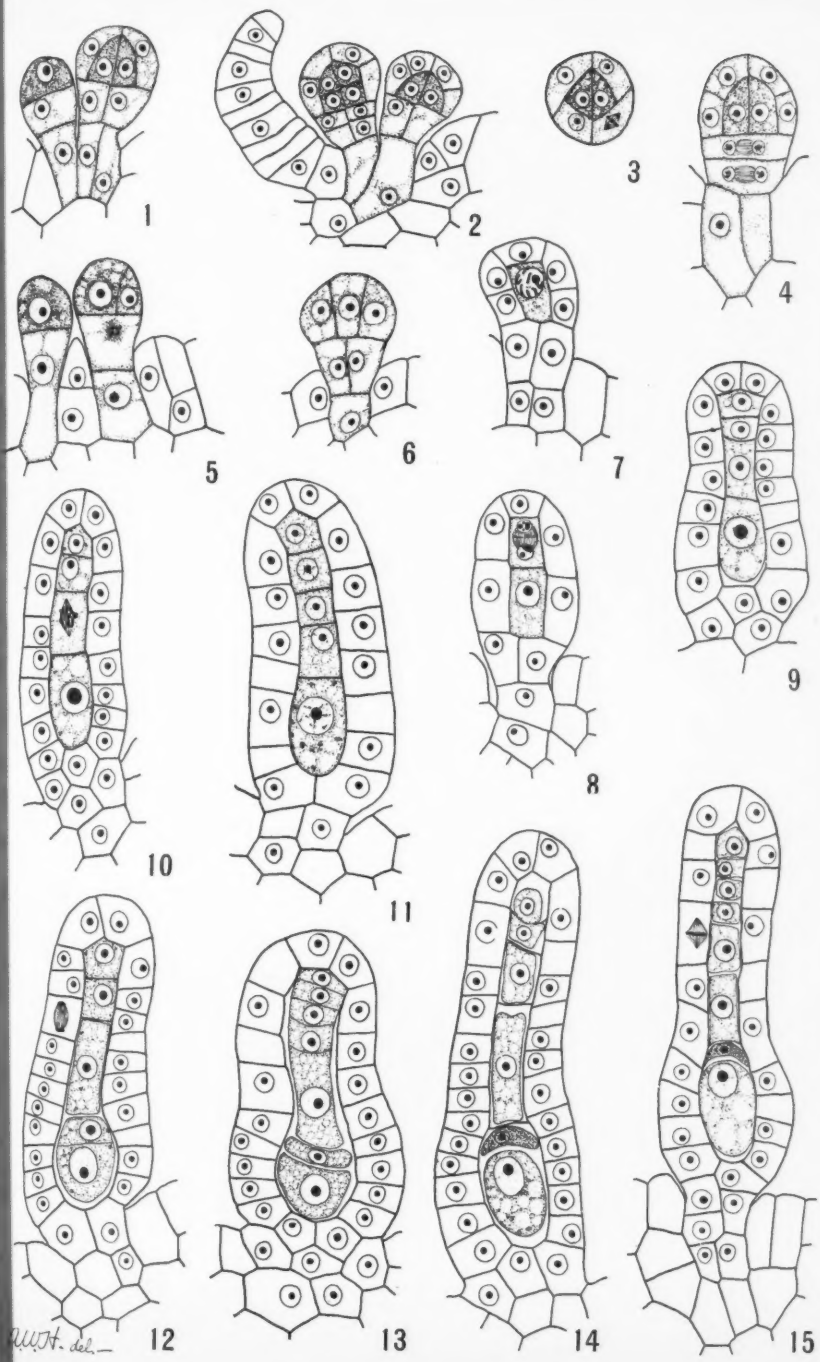
FIG. 11.—Young archegonium with axial row consisting of ventral cell and four neck canal cells.

FIG. 12.—Division of ventral cell to form ventral canal cell and egg, lower neck canal cell undivided.

FIG. 13.—Archegonium with lower neck canal cell still undivided, upper one having divided twice.

FIG. 14.—Archegonium with lower neck canal cell undivided, upper one having formed three.

FIG. 15.—Archegonium in which the lower neck canal cell has divided once, upper one twice.



HAUPT on HEPATICAE

BRIEFER ARTICLES

A CASE OF PHYLLODY IN YUCCA ELATA

(WITH TWO FIGURES)

Yucca elata Engelm., an evergreen of the family Liliaceae, occurs abundantly over the grasslands of southern New Mexico. In the late spring, after favorable precipitation, the plant produces a long flower stalk, surmounted by a great cluster of white or cream colored flowers, as shown in fig. 1. In observations made on the Jornada Range Reserve, a grazing experiment station in southern New Mexico conducted by the United States Forest Service, practically every plant of *Yucca elata* flowered during May or June of 1926. Later in that summer, several flower stalks were found with leaf clusters instead of flowers. In the summer of 1928 the excellent specimen of phyllody shown in fig. 2 was found on the Reserve. All the cases of phyllody have been found from one to two months after the regular flowering season for this plant. Observations to date have shown that such unusual flower stalks, as well as the ordinary ones, do not persist in a green condition longer than a year after production.—R. S. CAMPBELL, *Jornada Range Reserve, U.S. Forest Service, Las Cruces, New Mexico.*

FIG. 1.—*Yucca elata* in full bloom

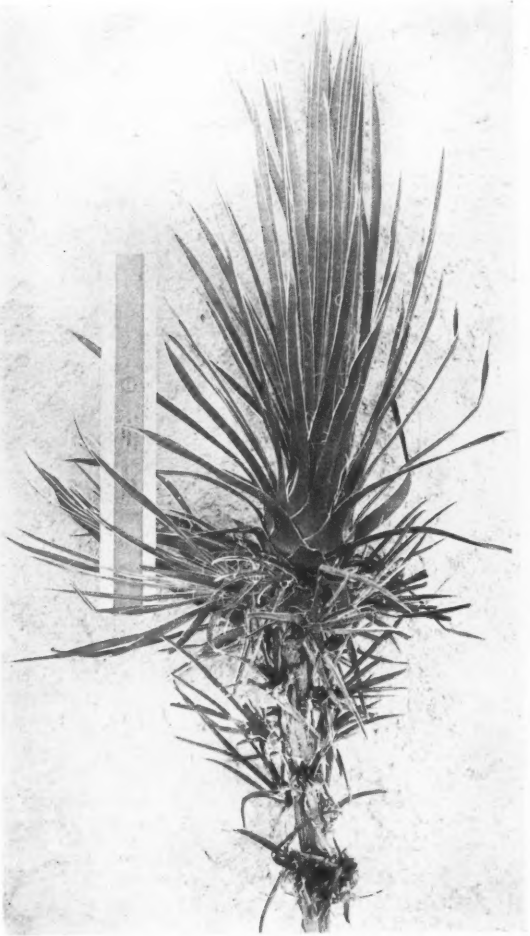


FIG. 2.—Phyllody in *Yucca elata*

CURRENT LITERATURE

BOOK REVIEWS

A moss flora of North America

American moss students have been at a considerable disadvantage in recent years because of the lack of an up-to-date general manual. LESQUEREUX and JAMES'S *Manual of the mosses of North America* (1884), supplemented by the BARNES and HEALD *Analytical keys to the genera and species of North American mosses* (1896), has long been out of date and difficult to obtain. Of the *North American flora*, only two parts have appeared dealing with mosses (1913), covering only nine families. This last work has no illustrations, is brief and rather technical, and because of its wide range (including all North America and the West Indies with many tropical mosses) the keys are larger and rather more difficult to use.

To supply the need for a general and not too difficult manual of all the mosses of the United States and Canada, GROUT, author of *Mosses with a hand-lens* and *Mosses with hand-lens and microscope*, has begun the publication of a moss flora of North America, north of Mexico. Part I of Volume 3 has appeared[1] (September 1928), including the Climaceae, Porotrichaceae, Isotheciaceae, and the Brachythecieae under the Hypnaceae. The families and genera are fully described, and there are keys to the genera and to the species. For each species there is given the more important synonymy, a full but not too briefly technical description, type locality, habitat, geographical range, citations to illustrations and to exsiccati, and usually critical and comparative notes. Varieties are described and line-drawn plates are included of all the mosses that have not been already illustrated in the author's *Mosses with hand-lens and microscope*, with which the moss flora agrees in size of page. The taxonomic sequence followed is that which GROUT has outlined in The Bryologist (31:56–61. 1928), and which differs considerably from that of the second edition of *Die natürlichen Pflanzenfamilien* (Band 10, Musci, 1. Hälfte, 1924, and Band II, 2. Hälfte, 1925).

This is an excellently and carefully prepared manual, the value of which is considerably enhanced by the good illustrations, and by the very interesting and helpful comparative and critical notes which accompany most of the specific descriptions. The reviewer ventures the opinion that the sequence used by GROUT will find favor with many American bryologists. Great service will be done American bryology by the publication of this manual, and it is to be hoped that it may be rapidly completed.—O. E. JENNINGS.

[1] GROUT, A. J., Moss flora of North America, north of Mexico. Vol. III, Pt. I. pp. 1–62. *pls. 1–14*. Published by the author, 1 Vine Street, New Brighton, Staten Island, New York City. 1928.

Manual of histochemistry

As a method of attacking the problems of chemical constitution of cells and tissues, histochemistry or microchemistry is recognized as an indispensable aid. It is also used in detecting the presence of soluble constituents in drug materials, and is rapidly becoming useful in the detection of the origin, transformation, and distribution of certain substances in the organism throughout life. Physiologists and pharmacologists need this discipline in much of their research.

To meet the need of a laboratory manual in this field, KLEIN[2] has prepared a little book which will be quite useful to the practicing histochemist. It does not in any sense take the place of such research literature as TUNMANN's *Pflanzenmikrochemie*, or MOLISCH's *Mikrochemie der Pflanze*, but it offers to students an introduction to the methods and purposes of microchemistry, with exercises in training for such work. Such a manual has long been needed.

The introductory chapter deals with the significance of histochemistry, the problems, the limits of its service, localization, the materials, reagents, instruments and utensils needed, hints on various types of reactions, histochemical and special methods. The second section gives tests for the inorganic substances, nine cations, and as many anions. Part III deals with the determination of organic substances, aliphatic, cyclic, nitrogenous, glucosidic, pigments, enzymes, membrane substances, and special inclusions of the cell. The final division, only seven pages, considers animal histochemistry. The directions are clear, and suggestions of appropriate plants for study are numerous. The student is aided by 64 text figures, mainly of crystals and other visible features of the histochemical reactions. It is recommended to students and instructors of students in microchemistry as an inexpensive laboratory guide in this important field of plant physiology.—C. A. SHULL.

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[2] KLEIN, G., Praktikum der Histochemie. 8vo. pp. v+71. Berlin: J. Springer, 1929.